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Prevalence of nuclear cataract in Swiss slaughter calves

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Für meine Eltern…

Table of contents

1 Summary

Purpose: To evaluate the prevalence and etiology of nuclear cataract in Swiss slaughter calves.

Material and methods: 253 freshly slaughtered calves were examined by slit lamp biomicroscopy. Lenses with cataracts were examined histological. The activity of glutathione peroxidase, catalase and superoxide dismutase was measured in aqueous humor of each eye. All calves were tested for BVD, Neospora caninum and Toxoplasma gondii. Since each calf in Switzerland is identified by an ear-tag, the complete pedigree was obtained. The correlation between congenital cataracts and the effects of non-ionizing radiation from mobile phone base stations (BS) was evaluated.

Results: 81 (32%) of the 253 calves showed nuclear cataracts of variable severity. Dense nuclear cataracts were seen in 9 calves (3.6%). None of the affected calves tested positive for BVD, Neospora caninum or T. gondii. Inherited cataracts could be ruled out based on pedigree analyses. More male calves were affected. Histology revealed signs of cataract in 62 of 100 lenses. The activity of glutathione peroxidase was significantly lower in aqueous humor of eyes with cataracts (p=0.03). An association of strength of BS with nuclear cataracts was shown to start in the first trimester of gestation.

Discussion: The prevalence of congenital nuclear cataracts in slaughter calves in Switzerland is relatively high. Method of choice for diagnosis is slit lamp biomicroscopy. Sensitivity of histology is low (62%).

The statistically significant decreased activity of glutathione peroxidase in eyes with cataract suggests that these eyes were under oxidative stress.

It appears that during organogenesis the risk for the development of nuclear cataracts in calves is associated with radiation of mobile telephone antenna BS.

2 Introduction

This study was initiated after an outbreak of spontaneous blindness in calves in a Swiss dairy farm, where 25% of the newborn calves showed severe nuclear cataracts. The first occurrences of nuclear cataracts on that farm coincided with the deployment of a mobile phone base (BS) station 30 meters from the cowshed, where the animals were kept. The influence of electromagnetic fields on public health is increasingly a matter of public concern due to the widespread use of mobile phone antennas in the last few years.

The objective of this study was to evaluate the prevalence and possible etiologies of nuclear cataracts in calves in Switzerland and to determine, whether there was an association between the strength and distance of mobile phone stations and the occurrence of cataracts. For this matter, known infectious causes for cataracts had to be ruled out.

According to the literature, the activity of the enzymes glutathione peroxidase, catalase and superoxide dismutase are valuable to evaluate a reduced redox status. The activities of these enzymes were measured and put into correlation with the respective results from the ophthalmic examination.

In Switzerland, an official ear tag identifies each calf. Central registration of all cattle movement allows the follow-up of each geographical location of every dam and calf from conception until slaughter of the calf. Each geographical position was put in relation to the strength and distance to the next mobile phone base station and all base stations within 10 kilometers.

3 List of abbreviations

BS	Base station
BVD/ BVDV	Bovine virus diarrhea/ Bovine virus diarrhea virus
cGPx	Classical glutathione peroxidase
DNA	Desoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
EMF	Electromagnetic field
GI-GPx	Gastrointestinal glutathione peroxidase
GPx	Glutathione peroxides
GSH	Glutathions
GSM	Global system for mobile communications
IFAT	Indirect fluorescent antibody test
IBR	Infectious bovine rhinotracheitis
K	Potassium
MD	Mucosal Disease
Na	Sodium
NADPH	Nicotinamid Adenin Dinucleotid Phosphate
N.caninum	Neospora caninum
OR	Odds ratio
PAS	Perjodic-acid-Schiff-Reaction
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
pGPx	Plasma glutathione peroxidase
PHGPx	Phospholipids hydroperoxide glutathione peroxidase
ROS	Reactive oxygen species
Se	Selenium
SOD	Superoxide dismutase
T.gondii	Toxoplasma gondii
UMTS	Universal mobile telecommunications system
UV	Ultraviolet

4 Literature

4.1 The lens

4.1.1 Anatomy

4.1.1.1 Introduction

The lens is a clear, uncolored, elastic and biconvex structure, located within the globe, behind the iris (Figure 1). Zonules, stretched between the ciliary body and the equatorial region of the lens, hold the lens in place. Contraction of the ciliary body muscles alters lens shape and thus the dioptric power in a process called accommodation. The posterior lens surface has a steeper curvature than the anterior surface [1-4].

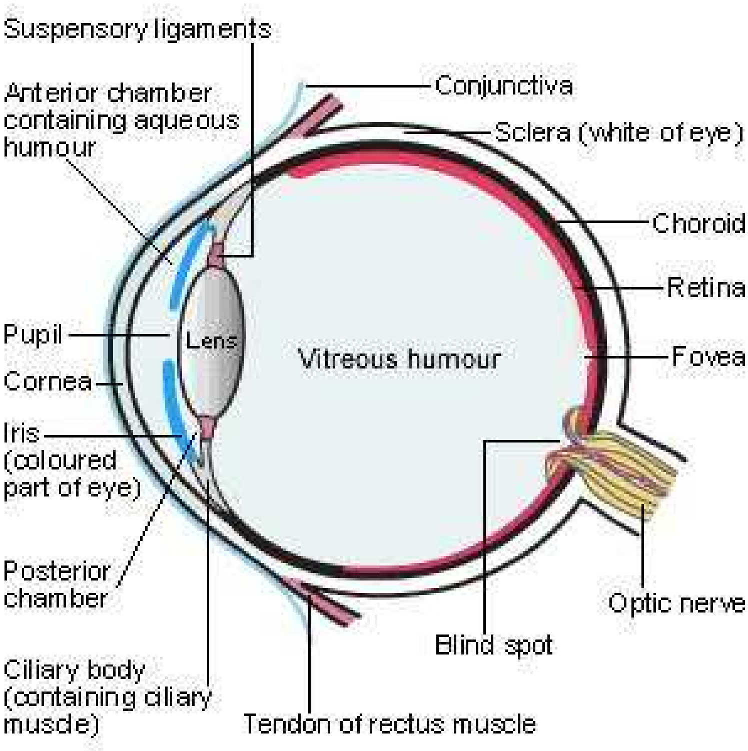


Figure 1: the eye (from http://www.mydr.com.au)

The lens is part of the refractive media of the eye and has a refractive power of approximately 40 diopters [4, 5]. This elastic structure must be transparent to be able to focus incoming light onto the retina and to accommodate. The healthy lens contains no pigment or blood vessels that would interfere with transparency. Due to this lack of blood supply, the lens is entirely dependent on the surrounding aqueous and vitreous humor for its metabolic needs [4, 6].

The lens of young mammals is quite soft with a small central nucleus. With age, the nucleus becomes denser, which reduces the ability of accommodation [3, 4].

Anatomically, the lens is divided in two principle structures: the central nucleus and the outer cortex [3] (Figure 2).

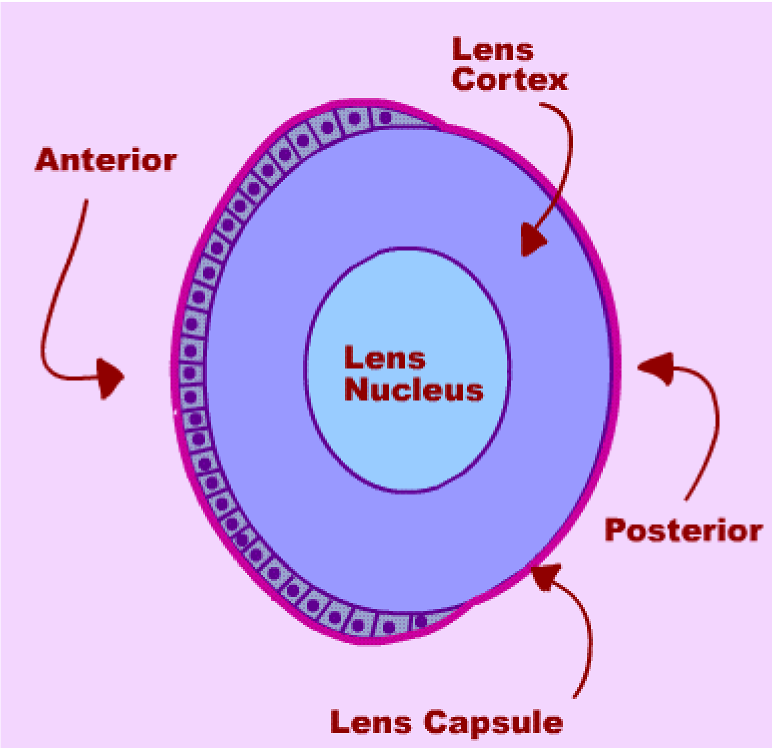


Figure 2: cross section of a lens (from http://www.dogstuff.info)

4.1.1.2 Lens capsule

The lens capsule is the basement membrane of the lens epithelium and consists of type IV collagen. Epithelial cells produce collagen fibrils, which are added to the cell membrane. The outer fibrils become more compressed, so that one to two months after birth, the capsule appears homogenous [4].

4.1.1.3 Lens cortex and nucleus

The lens epithelium, located under the anterior capsule, is the major site of metabolic activity in the lens [2]. Cells of the anterior lens epithelium divide throughout life (mitosis), become hexagonal, loose their nuclei, elongate to more slender lens fibers and move centrally, in direction of the nucleus [7]. Mature lens fibers have fewer organelles and therefore are highly dependent on the anterior epithelium cells for maintaining a critical level of dehydration. This dehydration increases the refractive index of the lens and provides a healthy level of reduced glutathione and keeps soluble lens proteins functional [2]. Throughout the entire life the lens continues to increase in cell number and size. While the fibers in the inner part of the lens are already produced in embryonic life, those in the subcapsular region of the cortex are only weeks or months old [7].

Every hexagonal fiber is in contact with six neighboring fibers. In most mammals the lens sutures, formed by concurring lens fibers, take the shape of a Y. At the anterior lens pole, the Y stands upright, at the posterior pole upside down [8].

The embryonic nucleus, located in the centre of the lens and consisting of primary lens fibers, is surrounded by the fetal nucleus, the adult nucleus (both of secondary fibers) and the cortex (consisting the youngest, secondary lens fibers) [9].

The anterior lens epithelium is very sensitive to many risks, like changes of O_2 concentration, the presence of toxins, X-rays and ultraviolet light. The last one could be the reason of age-related cataracts [10].

4.1.1.4 Lens transparency

Lens transparency is based on several factors: the nourishment of lens cells and capsule, the regular parallel arrangement of the lens fibers, the lack of cell organelles in the optical axis, the high concentration of soluble crystallines (80% of the lens proteins) in the fiber cytoplasm and the state of dehydration of the mature fibers [4, 7]. The lenses water content decreases with age [4].

Any opacity of the lens is called cataract. (See chapter 4.2)

4.1.1.5 Immunological role

As an entirely epithelial structure, lens proteins are separated from the immune system by the lens capsule. Immunotolerance against lens material never develops. If lens proteins leak out of the capsule, it is considered foreign protein and will stimulate antibody production. This will cause phacolytic or phacoclastique uveitis or phacoanaphylaxy [2, 11].

4.1.2 Embryology

Duration of gestation in cattle is on average 283 days [12]. The lens vesicle detaches from the surface ectoderm at day 2 [13]. Lens development in the bovine eye is completed by the end of the second trimester [14, 15]. The embryo is susceptible to teratogenic influences [16]. One important teratogene can be BVD.

4.1.3 Lens metabolism

4.1.3.1 Introduction

Maintaining a structure with unusually low water content and high protein concentration needs a continuous efflux of water, which continually enters the lens, especially from the posterior surface [2].

The main location of metabolism in the lens is located in the lens epithelium cells, which still have mitochondria [17, 18].

4.1.3.2 Protein

The lens contains 2/3 water and 1/3 structural proteins. Lens proteins form 35% of the lens volume, increasing up to 40% with the age, which is he highest proportion of all body tissues [4, 9, 19]. This high protein concentration is the cause of the high refractive index of the lens. The fibers closer to the lenses surface have a lower protein concentration than those in the inner part of the lens, which results in a gradient of the refractive index, which, in turn, partially corrects spherical aberration [2, 20]. Structural proteins are subdivided into soluble crystallines (α-, β- and γ-crystallines) and insoluble albuminoids [18].

With age, the insoluble portion of proteins increases and the soluble part (mainly α-crystallines) decreases, reducing elasticity of the lens [21]. This results in nuclear sclerosis, a normal aging process, which does not affect vision.

The concentration of insoluble proteins in the younger part of the lens is greater than in the older part. Soluble proteins are localized mainly in the lens cortex, whereas albuminoids are found particularly in the lens nucleus [9, 17]. β-crystallines form over half of the soluble protein fraction in the adult bovine lens [2].

Proteins are synthesized only in superficial fiber cells. Changes in protein composition lead to loss of enzyme activity (catalase, glutathione peroxidase), any changes of the structure of crystallines, cytoskeletal proteins and enzymes can lead to cataract formation [7].

4.1.3.3 Lipids

The lenses total lipid content is about 2.5% of the wet weight and consists mostly of phospholipids in the fiber cell membranes [2]. In human senile cataract, a lipid-degradation has occurred in this phospholipid fraction in the inner cortex and nucleus, which is consistent with membrane disintegration.

4.1.3.4 Energy metabolism

The lens capsule is a semipermeable membrane, only permeable for molecules with a low molecular weight [3, 17, 18]. It maintains its clarity only as long as it is surrounded by aqueous humor for supply with nutrients [4, 17]. Of the four important metabolic pathways, three are soluble in the cytosol: anaerobic glycolysis, the pentose phosphate pathway, and the sorbitol pathway. The fourth metabolic pathway, the aerobe citric acid cycle, is insoluble and found only in mitochondria of young cells [2].

The bulk of the required energy is supplied by anaerobic glycolysis [22]. Glucose enters the lens by facilitated diffusion. There appears to be evidence that the lens possesses a specific glucose transporter [23]. Aerobe glycolysis provides 20% of the lenses energy and is only possible in lens epithelium cells, which still have a nucleus. Cells near the lenses surface may use both, aerobic and anaerobic glycolysis [9].

The major cations of the lens are potassium and sodium. Potassium is used for different enzyme reactions in the cells. Sodium, in contrast, is extruded from the cell. Sodium concentration decreases towards the lens nucleus. Sodium and potassium continually enter into the cell from the posterior surface using an ATP-dependent active transport mechanism facilitated by glycolysis [2].

When lactate, an end product of anaerobe glycolysis accumulates, intracellular pH-level decreases significantly from peripheral to central lens fibers [24]. This is the reason why pH-sensitive processes are affected differentially in different regions of the lens [25]. Water extrusion is important for the maintenance of the transparency of the lens. Water follows sodium, but a current new concept discusses also a metabolic water pump existing in the lens. Failure of water or sodium removal out of the lens will lead to cataract formation [2].

4.1.4 Oxidative Stress

4.1.4.1 Oxidative stress in the lens

The lens, like all biological systems, depends on a balance between oxidation and reduction [18, 26]. Free radicals and molecular oxygen can cause oxidative stress and therefore reduce transparency. Molecular oxygen is generated by mitochondria, by metabolic processes and by light absorption. To prevent oxidation, cells try to maintain a reducing environment in the cytoplasma. The majority of cell fibers have no contact with the epithelium or the capsule, which calls for a delicate balance between nutrition and antioxidative protection [26].

Reactive oxygen species (ROS), such as hydroxyl radicals, superoxide anions and nitric oxide are produced during normal cell function. Hydrogen peroxide, produced in mitochondria by superoxide dismutase is also responsible for oxidative stress in the lens. Hydrogen peroxide is not a strong oxidant, but in presence of ferrous anions, it produces free radicals [2].

ROS can be generated endogenously by enzymatic systems (in mitochondria, peroxisomes, et cetera) or exogenously (ultraviolet light, ionizing radiation, chemotherapeutics, inflammatory cytokines, environmental toxins) [10, 26]. It has been estimated that a human cell is exposed to about 1.5×10^5 oxidative hits a day from reactive oxygen species [27]. Antioxidative enzymes maintain normal conditions in the cell and form the first line against oxidative stress by reducing free radicals and molecular oxygen [26, 28]. Thus, there is a balanced redox state in the cell. Any imbalance in favor of oxidants results in oxidative stress, which can end in lipid peroxidation, enzyme oxidation, DNA damage and protein modification [10, 29, 30]. The results can be oxidative stress-associated diseases like cancer, cardiovascular diseases, nerve degenerations, macular degeneration and cataracts [31-33]. Although the lens is exposed to light during the entire life, there is no evidence for photo-oxidation of proteins in the central region of the lens [34, 35].

Antioxidative enzymes are superoxide dismutase, glutathione peroxidase and catalase. Vitamin C (ascorbic acid), E (α -tocopherol) and A (retinol) are nutritional antioxidants [26, 32, 36, 37] (Figure 3).

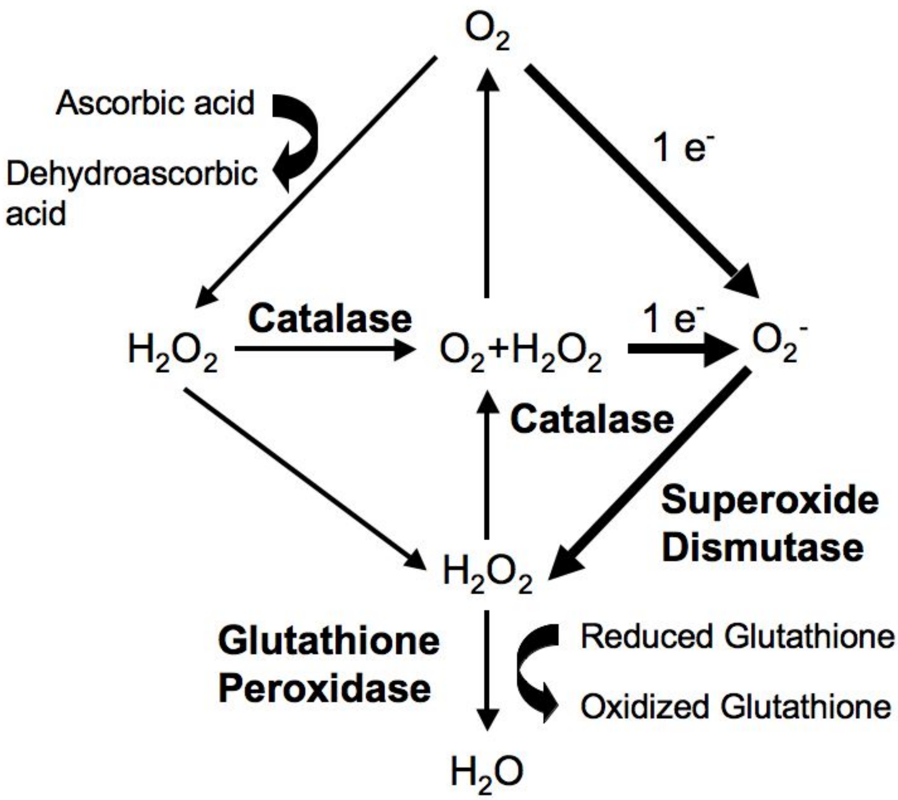


Figure 3: oxidative stress

4.1.4.2 Superoxide dismutase (SOD)

Superoxide dismutase acts as the first enzyme in the pathway of oxygen reduction [38, 39]. In mitochondria of lens epithelium cells, it produces hydrogen peroxide (H_2O_2) from free radical superoxide as a by-product of oxidative phosphorylation. In fact, hydrogen peroxide is not a radical, but it is reactive and therefore metabolized by catalase or glutathione peroxidase [38, 40]. If not, H_2O_2 will rapidly convert into OH^-, a highly reactive molecule [38]. SOD is significantly decreased in human lenses with advanced senile cataract [41].

SOD converts superoxide to hydrogen peroxide and molecular oxygen [42]:

$$O_2^- + 2\,H^+ \rightarrow H_2O_2 + O_2$$

4.1.4.3 Glutathione (GSH)

Glutathione is present in high concentrations in the lens and plays an essential role in the lens metabolism as a thiol antioxidant [43-46]. It is the most important component of the enzyme glutathione peroxidase and is involved in cell protection as antioxidant or as cofactor of glutathione peroxidase [44]. Cell damage and cataract formation results, if glutathione concentration in the lens epithelium cells is decreased [45, 47]. In all types of cataract, there is a decline of glutathione content in the lens [26, 43]. Glutathione is transported into the lens from aqueous humor or synthesized from lens epithelial and superficial fiber cells [45, 48]. Thus, there is a concentration gradient of GSH into the lens, with the highest level in the outer layer and a gradual decrease towards the nucleus [26].

Glutathione reductase and NADPH regenerate reduced glutathione (GSSG) [45]. High GSH content in cells is a major reason for reliable resistance to oxidative stress [49].

4.1.4.4 Glutathione peroxidase (GPx)

Glutathione peroxidase connects the reduction of hydrogen peroxide to the oxidation of glutathione [50]. The enzyme is present in the cytosol as well as in mitochondria [38]. Selenium is part of the active site of glutathione peroxidase [2, 50]. Four mammalian selenoproteins form the glutathione peroxidase family (Table 1). CGPx, the classical glutathione peroxidase is ubiquitously distributed and has its main function in the defense of oxidative attacks. It can metabolize hydrogen peroxide and a range of organic peroxides [51]. In the gastrointestinal tract, GI-GPx provides a barrier against hydroperoxides derived from dietary intake or metabolism. GI-GPx has the highest stability against selenium deficiency of all glutathione peroxidases. CGPx and GI-

GPx both reduce hydrogen peroxide or fatty acid hydroperoxide rapidly, but not phospholipid hydroperoxides. In kidney, ciliary body and maternal/fetal interfaces, plasma-GPx (pGPx) is expressed as an efficient extracellular antioxidant but with low capacity. PGPx is also secreted into aqueous humor [52]. PHGPx, the phospholipids hydroperoxide glutathione peroxidase, is the only antioxidant enzyme to protect membrane lipids [53]. It can use a wide range of reducing substrates as well as glutathione [54]. There are also many selenium-independent GPx activities in mammalian systems, which are mainly associated with the glutathione-S-transferases. Those have important activity against organic hydroperoxides and little activity towards hydrogen peroxides [55].

Table 1: Selenium-dependant glutathione peroxidases [50]:

GPx1	Cytoplasmatic glutathione peroxidase
GPx2	Gastrointestinal glutathione peroxidase
GPx3	Plasma glutathione peroxidase
GPx4	Phospholipid hydroperoxide glutathione peroxidase
GPx5	Function unknown yet
GPx6	Eventually a homologous of GPx1

Glutathione peroxidase uses reduced glutathione (GSH) as a substrate to transfer electrons to H_2O_2, converting it into water [38]:

$$2\ GSH + H_2O_2 \rightarrow GSSG + H_2O$$

Glutathione peroxidase is the most effective enzyme against oxidative damage due to hydrogen peroxide in the lens, particularly in low levels of oxidative stress. Lenses standing under oxidative stress show low GSH levels because of oxygen-susceptible sulfhydrylgroups [26].

Catalase (See Chapter 4.1.4.6) in contrast, is only effective against high concentrations of peroxides [46, 56].

4.1.4.5 Selenium

Selenium is a micronutrient and essential for immunity and protection against oxidative damage [57]. Selenium and vitamin E seem to have similar antioxidant activities [57]. Blood selenium is divided into [58]:

1) Selenium as a component of amino acids and therefore proteins and cell membranes

2) Selenium as part of glutathione peroxidase

3) Only in very small concentrations, as anorganic selenium.

Selenium absorption in the gastrointestinal tract in ruminants is less effective than in monogastrians, because of the reductive environment in the rumen [5]. The activity of glutathione peroxidase depends on the selenium intake [59]. Only under standardized feeding conditions, is measuring of selenium concentration by means of GPx activity in erythrocytes reliable [60]. Determination of glutathione peroxidase activity seems to be the more sensitive method than measuring the selenium concentration in tissues for monitoring selenium intake of an animal. Selenium-dependant GPx plays an essential role in modulation of the inflammatory response [61].

Information concerning selenium concentration in aqueous humor is variable. In a veterinary study of the North Carolina State University, mean selenium concentration in aqueous humor of healthy eyes was 0.008 μg/ml, much lower than that of plasma (0.21 μg/ml). In plasma, selenium is bound to proteins, therefore it is likely, that the difference in selenium concentration between plasma and aqueous humor reflects the relative distribution of protein between these two fluids [62]. Selenium concentration in a human study was 0.19 μg/ml in aqueous humor of patients with senile cataract, what is suspected to reflect defective antioxidative systems during cataract formation [63].

4.1.4.6 Catalase

Catalase is a tetrameric haemin-enzyme consisting of four identical subunits and is present mainly in the peroxisomes [38].

In galactosemic cataracts, induced in albino rats, a decreased catalase-activity has been proven [64]. In a human study, plasma catalase levels in patients with cataract were significantly lower than in control patients [65].

Catalase converts hydrogen peroxide into water [40].

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$$

4.1.4.7 Regulation of antioxidant enzymes

Multiple factors influence the antioxidant level of a cell. The oxidative state of the cell dictates gene expression and activity of antioxidant enzymes [66, 67]. Inflammation and aging influences as well as hormonal regulation of antioxidative enzymes have been reported [68, 69]. In addition, several antioxidants and cell protectors are believed to regulate gene expression and antioxidant enzyme activity [38, 70, 71].

When cells are exposed to oxidative stress, activity and expression of antioxidant enzymes increase to protect the cell from oxidative damage. But even the increased enzyme activity can be insufficient to counteract free radicals. Under highly elevated oxidative stress conditions, decreased enzyme activities can be found. Moderate levels of free radicals appear to induce high antioxidant levels, while high levels reduce enzyme activities as a result of damage to the molecular machinery, which normally initiates these enzymes [38].

4.2 Cataract

4.2.1 Introduction

Any opacity of the lens or its capsule, which prevents light of reaching the retina, is called cataract. It may clinically be diagnosed by slit lamp biomicroscopy. Depending on their specific structure, cataracts cause reflection, refraction or dispersion of light [1, 72].

It is important to distinguish between the ophthalmologic definition of cataract, where all opacities in the lens are called cataracts and the clinical definition, where only opacities with reduction of vision are called cataracts. Loss of lens transparency can be caused by an increase of light absorption or light scattering [7].

Cataracts have various etiologies and the cause often remains unknown. It can be categorized as primary or secondary. Primary cataracts are not associated with concurrent or antecedent ocular or systemic disease. They may be congenital or developmental. Secondary cataracts are the result of trauma, or systemic and/or ocular disease, such as metabolic, inflammatory or infectious diseases, toxic insults, radiation, et cetera.

Many cataracts may be the result of local accumulation of water; others may be caused by formation of crystals, by regional pressure, or by extremely large aggregates of lens proteins of molecular weight ≥ 50 million Daltons. These proteins, although still soluble, cause light scattering, because their size approaches the wavelength of visible light [2].

True cataracts have to be distinguished from nuclear sclerosis, a physiologic aging phenomenon, which is not blocking light from reaching the retina.

The healthy lens it totally transparent. This transparency has several reasons:

1. Low cytoplasm density in the epithelial cells and fibers
2. Lack of intracellular organelles in lens fibers
3. Parallel arrangement of lens fibers
4. High fraction of soluble proteins
5. Relative dehydration of the lens

Alteration of one of these factors will mark the beginning of cataractogenesis [3, 7].

4.2.2 Cataract classification

Table 2: cataract classifications (according to D. Slatter, Lens [3])

Stage of development	Incipient, immature, mature, hypermature, intumescent, morgagnian
Position within the lens	Capsular, anterior subcapsular, posterior subcapsular, cortical, nuclear, posterior polar, axial, equatorial
Time of development	Embryonic, congenital, developmental, juvenile, senile
Appearance	Brunescent, cerulean, coronary, corolliform, cuneiform, cupiliform, discoid, floriform, fusiform, membranous, punctuate, pyramidal, spear, stellate, sutural
Etiology/ Pathogenesis	Primary, secondary (traumatic, radiation, diabetic, galactosemic, electric, toxic)
Consistency	Fluid, soft, hard

4.2.2.1 Congenital cataracts

4.2.2.1.1 Introduction

Congenital cataracts are present at birth, but have their origin in fetal life. Eyes with congenital lens anomalies often exhibit multiple ocular defects, including microphthalmia, anophthalmia, persistent pupillary membranes, retinal dysplasia, and others [73]. In calves and foals, they are seen before two weeks of age. In small animals, congenital cataracts may be noticed when they open their eyes. The cause can be inheritance, or it can be the result of a disturbance in secondary development, or of maternal influences. Most of the congenital cataracts are not inherited, but genetic factors can be involved [74].

4.2.2.1.2 Inheritance of cataract

Congenital cataracts are not necessarily inherited. Inherited cataracts have been reported in several different species [75-77]. To prove evidence of inheritance of cataracts, pedigree analyses have to be performed.

4.2.2.1.3 Congenital cataracts caused by environmental factors

Congenital cataracts may also be of maternal origin, resulting from exposure to a infectious or toxic agent, or intrauterine dietary deficiency [78, 79].

4.2.3 Histopathology of cataract

Any noxious stimulus can produce a dysfunction of the osmotic metabolism, which will result in swelling, pyknosis and karyorrhexis of lens epithelial cells, followed by formation of fluid-filled clefts and vacuoles. Lytic lens proteins leak from the lens fibers and so the fibers shrink. Fluid collects in the clefts, cell membranes disintegrate, and fiber protein gets released. Intercellular fluid becomes albuminous and looks like eosinophilic material clefts. The clefts may be associated with vacuolization of the lens fibers and pyknotic nuclei [1]. Larger clefts with protein degenerative material may be seen as small eosinophilic aggregates or Morgagnian globules. Then, protein coagulates and permanent opacity of the lens results [80]. Due to degeneration of cortical lens fibers, the lens epithelium becomes hyperplastic, and the basal membrane can change into plaque-like, fibrous or dystrophic calcification [1].

In the subcapsular regions of a cataractous lens balloon-like, swollen, so called Wedl-cells or “bladder”-cells are found [1].

4.2.4 Metabolic cataract formation

Lens biochemistry is very complex. There are numerous causes for cataracts. The exact biochemical processes in cataract formation are still not fully understood, with the exception of diabetic, galactosemic and experimental cataracts [3].

Any insult affecting the following lens metabolisms may result in opacity:

1. Lens nutrition
2. Energy metabolism
3. Protein metabolism
4. Osmotic balance

Looking at the protein fraction in cataractous lenses, the high-molecular-weight insoluble proteins (albuminoids) have increased and the soluble crystalline proteins have decreased. Also the Na/K-ATPase-activity has decreased, which explains the imbalance of electrolytes and therefore osmotic pressure.

In contrast, the activity of hydrolytic and proteolytic enzymes has increased, causing cell membrane rupture and influx of water. The lens derives most of its energy from glycolysis, with the end product of lactic acid. Lactate accumulation leads to a decreased intracellular pH from peripheral to deeper lens fibers. This is the reason why pH- sensitive processes are differentially affected in different lens regions [24, 25, 81].

Later, the proteolysis-products (amino acids, polypeptides) have to diffuse from the lens, which results in loss of water and electrolytes, which causes the lens to shrink (hypermature cataract) [3].

Senescent, cortical cataracts in humans manifest more by derangement of electrolyte and water balance, whereas nuclear cataracts are primarily associated with protein modification and insolubilization.

Cataracts may be bilateral or unilateral. Congenital cataracts are often bilateral [3].

4.2.5 Cataract and oxidative stress

Oxidative stress has been suggested to play a major role in many ocular diseases, such as cataracts and macular degeneration [82]. Cataracts are often associated with decreased activity of the lens epithelial Na/K-ATPase- pump. This in turn may cause a decrease in potassium content and an increase in calcium and sodium levels within the lens. ATP and decreases and antioxidant activity is diminished, including decreased levels of glutathione, vitamin C and vitamin E and superoxide dismutase [1].

The lens has a well-designed system of defense against oxidation [83]. Antioxidants like non-enzymatic (glutathione, vitamin C, vitamin E, carotinoids) and enzymatic (SOD, GPx, catalase) systems prevent the lens from oxidative stress. In case of protein damage due to oxidation, lens cells contain enzymes, which degrade these proteins through proteolysis or nucleic acid repair [33, 84]. Under oxidative stress, these defense systems can be upregulated.

If these defense systems are downregulated or overstrained because of oversupply of free radicals, damage results. The damage due to reactive oxygen species in the lens consists of protein modification, lipid peroxidation and DNA fragmentation. All of which have been proposed to contribute to cataractogenesis [26, 33, 85].

A high intake of antioxidants like carotenoids, vitamin A, vitamin E and vitamin C is associated with a lower risk of cataract [86]. Several mechanistic studies in ocular model systems have suggested that antioxidants can protect lens proteins against oxidative damage [87]. Glutathione peroxidase activity in the lens is decreased and the content of free radicals is increased in rats fed a selenium-deficient diet [88].

Three case-control studies failed to show any correlation between plasma selenium concentration and cataract [89-91]. No difference in plasma selenium concentration or glutathione peroxidase activity has been found in people with or without cataract [92]. PGPx seems to have similar activity in the different types of cataract [52].

The level of cGPx in aqueous humor is just 18% of that in serum. Serum selenium has a positive correlation with serum cGPx and aqueous humor cGPx, but in a study by Huang (1997), there seemed to be no significant correlation between cGPx levels in aqueous humor and serum. This may indicate that secretion or maintenance of cGPx in this two fluids is controlled by other factors beside selenium [52].

Fecondo (1983) observed no changes in the activity of catalase with the progressive development of cataract in human, calf, rabbit and rat lenses. Superoxide dismutase and glutathione peroxidase activity decreased at 70% at the onset of nuclear cataract formation. This shows that the inactivation of these antioxidative enzymes may result in an elevation of H_2O_2 and O_2 levels in the lens, which in turn results in oxidative stress and, consequently, modification of lens proteins, as observed in nuclear cataracts [93, 94].

In the nuclear region of the lens, a protein content of over 50% is found, mostly structural proteins like crystallines. Crystallines need to stay in a reduced state in order to maintain lens transparency [95]. As the lens ages, GPx becomes less efficient and GPx concentration declines [96]. In rats and humans, a progressive loss of GPx in the aging lens could be demonstrated and as a result the risk for cataracts is increased [97].

In a recent study, Barros et al. (2004) showed that the activity of superoxide dismutase and catalase in 14 noncataractous poodles was significantly higher than in 15 cataractous poodles. Glutathione peroxidase activity, on the other hand, was constant [98].

4.2.6 Age related cataracts

Age-related cataracts can be nuclear, cortical and posterior subcapsular. Nuclear cataracts occur in the oldest fiber cells, those formed during embryonic and fetal life. Cortical cataracts occur in fiber cells formed later in life. Light scattering by a plaque of swollen cells migrated to the posterior pole of the lens results in posterior subcapsular cataract [7].

4.2.7 Nuclear Cataract

It appears to be obvious, that increased oxidative damage to lens proteins and lipids is resulting in nuclear cataract [33, 45, 99]. This was also evaluated in a convincing study, where patients have been treated with hyperbaric oxygen to alleviate the complications of peripheral vascular diseases [100]. An association between oxidation, age and cataract formation was obvious. The fact, that hyperbaric oxygen results in opacity of the lens nucleus but not in peripherals parts of the lens, demonstrates, that central lens fibers are more susceptible to oxidative damage. The low diffusion rate of glutathione into the lens could be the reason for this increased susceptibility [45, 101]. Lens fiber cells in the nucleus are in a delicate balance between their ability to prevent or reverse oxidative stress and the tendency for oxidation to occur.

Nuclear cataract formation is associated with an increased light scattering in the nuclear fiber cells. This can be caused by separation of the lens cell cytoplasm into protein-rich and protein-poor liquid phases or by protein aggregation [102, 103]. Protein phase separation can be caused by alteration of the soluble proteins in the nucleus or by modification in the ionic composition of the solvent phase in this region. It seems to be evident, that phase separation can result in cataract formation [104, 105]. In contrast, experimental studies showed that extensive phase separation is not detectable in some nuclear cataracts [106]. Changes in the organization of proteins, which lead to cataract formation, seem to be subtle, despite of dramatic change in the hardness of the lens nucleus during nuclear cataract formation. It is interesting to note, that even if only a small fraction of lens protein in the fiber cell cytoplasm is aggregating, the result can be a dramatic increase of light scattering [107].

When aging, maintaining the cytoplasm in a reduced state under oxidative stress conditions is more difficult [108]. Any increase in the oxidative load or decrease of the lens nucleus to handle the normal level of oxidation is likely to cause nuclear cataract. High oxygen load in the bovine lens has a toxic effect and leads to a decrease in enzymatic activities and lens transparency [109, 110].

4.2.8 Posterior subcapsular cataract

Any opacity in the optical axis, at the posterior pole of the lens right underneath the capsule, is termed posterior subcapsular cataract. The scattering is caused by a cluster of swollen cells and the most superficial fiber cells are disorganized [111]. Opacities can also occur as “sutural” cataracts, a swelling of the posterior ends of the fibers along the suture planes at the posterior pole of the lens. These sutural opacities are typical for inherited cataracts and are not common in age-related cataracts. The cell biology of posterior subcapsular cataracts has been studied in experimentally induced radiation cataracts in amphibians and rodents [112, 113]. Humans develop posterior subcapsular cataracts during interventional radiotherapy [114].

4.2.9 Radiation cataract

β-particles, neutrons, γ-rays, X-rays, UV-light, infrared light and microwaves can cause radiation cataract [115]. X-ray cataract is the best described, the effects of neutrons and γ-rays seem to be identical [2]. Susceptibility to damage caused by X-ray is closely related to the mitotic rate of the epithelium, which decreases with age. Therefore, young lenses are more sensitive to X-rays than older lenses. Hence, radiation during lens development seems to have much more consequences.

Cataracts can be caused by exposure to electromagnetic fields. Ionizing radiation is the best studied for cataract [116]. In experimental animals, x-/or γ-radiation causes posterior subcapsular and cortical cataracts. The initial cataractogenic insult may be damage to the proliferating cells in the germinative zone of the lens epithelium. This leads to extensive cell death in this region [112, 117, 118]. Cell death in the germinative zone is first followed by a decreased cell division, and then by a wave of compensatory mitosis, which results in a disrupted organization of the fiber cells. The nuclei of the elongating cells move posteriorly, and the abnormal fiber cells may form "balloon cells" at the posterior pole, which results in formation of posterior subcapsular cataract. There is evidence of increased membrane permeability during this process. Glutathione and potassium levels decrease in the cytoplasm, whereas sodium concentration increases and the protein synthesis slows down [119, 120]. Ionizing radiation is a risk factor for cortical and posterior subcapsular cataract formation in humans. Non-ionizing radiation can cause cataract as well. Epidemiologic studies link high lifetime exposure to UV light to the formation of cortical cataract in humans [121, 122].

Some studies suggest that protection against free radicals and their effects might reduce the incidence of cataract formation [123]. Paradoxically, the cells that are affected by UV light are the best protected, as they lay behind the normally pigmented iris. Cortical cataracts form in superficial fiber cells, but those cells in the centre of the lens are usually not damaged by exposure to UV light [35]. In addition to UV light, long-term exposure to infrared light as well as focused, high-energy microwaves can result in cataract formation [124].

4.2.10 Other causes for cataract

Secondary cataracts in are the result of ocular or systemic disease, such as IBR virus or malignant catarrhal fever. Infection with Rhizopus sp. has also been incriminated [125]. Vitamin A deficiency has found to establish cataract in calves [126].

High-dose steroid intake carries the risk for formation of posterior subcapsular cataract in humans [127]. There are few animal models supporting this theory [113, 128].

A well-known cause for cataracts is diabetes mellitus, which starts as an equatorial opacity, most likely caused by the enzyme aldose reductase, which catalyzes the reduction of aldehydes, including glucose and galactose. A high level of aldose reductase appears to result in a higher risk of diabetic cataract formation, as demonstrated by the accumulation of the polyol sorbitol in the lens fiber cells [129]. This accumulation can lead to osmotic damage and the high glucose flux through the polyol pathway could end in oxidative stress in lens fiber cells. Human lenses have relatively low levels of aldose reductase, which questions the role of this particular enzyme in human diabetic cataracts.

Epidemiologic studies show other risk factors for cataract in human lenses, such as lower socioeconomic status or low education level, probably reflecting nutritional deficiencies, increased exposure to diseases, and poorer health status. But the primary risk factor is the age. Cataract incidence is increasing after the age of 50 years. Women have a higher risk for cataract than men, due to decreasing level of estrogen during menopause [130, 131]. Alcohol is described in some studies as risk factors for nuclear and in some cases cortical cataract. The discussion about smoking as a risk factor is controversial [89, 132].

4.3 Ocular disorders in calves

4.3.1 Introduction

Acquired ocular disorders in cattle occur much more often than congenital diseases [133]. There are only a few reports about the incidence of eye diseases in food animals. In 1968, 502 Brown Swiss cattle in Denmark were examined, 18.8% showed ocular variations. The incidence for ocular abnormalities was increasing with age, starting at 3% in cattle less than 6 years of age, to 43% in cattle between 7 and

14 years, and finally to 75% in cattle at an age of more than 14 years [134]. An other study showed an 14.6% incidence of ophthalmic lesions in 1100 slaughter cattle in Queensland [135]. Herefords were significantly overrepresented, regarding neoplastic and inflammatory disease, cataracts were not described.

The incidence of congenital ocular defects in cattle was reported by Priester (1972), Greene (1973) and Schade (1974) and was between 2.9 and 55.9% [136-138]. None of them mentioned the method of examination.

4.3.2 Cataract in calves

In most studies about cattle, cataracts have been reported in association with other ocular anomalies or as a primary disease. Bilateral congenital cataracts have been described in Jersey, Holstein and Hereford breeds. In Holstein, Jersey and Shorthorn breeds, cataracts have been seen with other ocular diseases like lens luxation, buphthalmos, microphthalmia, retinal detachment and rupture of the lens capsule [133].

Odorfer (1995) published a study in which 31% of the calves of 22 weeks and 18% of adult cattle in Austria had cataracts. In 28% of the calves and in 18% of the adults, the cataract was nuclear [139]. Congenital cortical cataracts have been previously reported in 1971 [140].

Examining the congenital syndrome of anophthalmia/microphthalmia in 26 cattle, collected in a 15-year period, Leipold (1968) did not find any evidence of cataracts. The dams of many of the affected calves were aged [141].

Ashton (1977) investigated two herds of Friesian calves [142]. In the first herd, 59 of 192 calves (30,7%) were born with cataracts. Male and female calves had both been affected in equal numbers. Neither the dams nor the bulls were affected themselves. In the second herd, 48 of 140 calves had opacities in their lenses (34.3%). Again, both sexes were equally affected. The cataracts were always nuclear and bilateral, but of varying severity. Cortices were always perfectly clear, so cataracts were never affecting the entire lens. Several facts suggested an environmental rather than a hereditary etiology. An interesting fact was, that in cows inseminated between September and December the incidence of calves with cataracts was higher. Clay described 1977 that in calves born in late summer or born to heifers, cataract incidence was higher [143].

822 calves aged between one day and six months have been examined in 1996 and 1998 in Japan. 3% had ocular lesions, which included seven cases with cataracts (0,85% of the calves). Only one of the calves had a nuclear cataract. The lesions were characterized histological by degenerative changes in the lens fibers and the appearance of eosinophilic globules known as Morgagnian globules [144].

4.3.3 Congenital cataracts in calves

4.3.3.1 Introduction

Congenital cataracts are abnormalities of structure or function of the lens, present already at birth and are often associated with other ocular defects [16, 133, 145]. Diagnosis depends on the nature and extent of the defect. Many defective neonates are either not reported or escape monitoring systems [16]. Congenital cataract can be caused by intrauterine infection during pregnancy or has a hereditary basis. Usually the cause remains unknown. Pedigree analysis may be promising. “Congenital” and “genetic” should not be used synonymously, because not all defects present at birth are caused by genetic factors, but also by environmental factors [16].

Congenital cataracts have already been reported in several breeds of cattle in the early 19th century. Hereford, Holstein-Friesian and Jersey cattle have been examined and in purebred Jersey a simple autosomal recessive trait has been suggested [146, 147]. Autosomal dominant inheritance has also been reported in Jersey breed [148].

Priester found one out of 27 cattle affected with congenital cataract [137]. Schade found congenital cataracts in calves to be located in the subcapsular lens cortex. He saw variable numbers of congenital cataracts in different breeds of cattle reaching from 2.9% in Jersey to 55.9% in Holstein cattle [136].

Congenital bilateral cataracts appear to be rare in cattle and are often associated with other eye anomalies or diseases of the nervous system [149]. Some calves are blind, while others have varying degrees of vision. Congenital blindness was reported in 147 Brown Swiss calves 1974 in Switzerland [150]. Sixteen of these calves were examined ophthalmologically. All calves had bilateral cataracts, 12 of them had also a small corneal opacity (10 mild, 2 severe opacities). In addition, three calves had a congenital shortening of their tail. The author pointed out that when examining calves with cornea opacities, cataracts could be missed.

4.3.3.2 Congenital cataracts caused by environmental factors in calves

In 1970, Ward published a case report regarding a dairy cattle herd, where he found common fetal death [151]. One calf was blind, ataxic, weak and exhibited nystagmus, when placed on its back. Post mortem examination of the calf showed a very thin carcass, a small cerebellum and extensive opacity of the cornea in both eyes. The calf tested positive for BVD. No abnormalities were found in the lenses.

The problem of natural or experimental (between day 102 and 183 of gestation) BVDV-infection of diary cattle was examined by Kahrs (1970) [152]. Three out of four experimentally infected and two out of four naturally infected cattle showed lens opacities (cortical cataracts) and visual defects. The cumulative occurrence of cerebellar hypoplasia was also of interest. BVD infection as an etiology for cataract in calves was also the subject of a study by Bistner, published in 1970 [153]. He associated microphthalmia, cortical cataracts and retinal lesions in newborn calves with in uteri infection with BVDV at days 76 to 159 of gestation. Thus, the resulting defects depend on the stage of gestation at which the dam is exposed.

France (1990) published a study about a herd of 60 Friesian cattle in Somerset England. In 1985, approximately 90% of the calves were born with a severe and ophthalmoscopically visible cataract. The herd was otherwise healthy. In 1986 20%, and in 1987 40% of the calves were affected. In 1987, blood samples from 30 cows were taken with no result regarding to glucose, calcium and urea concentration in blood samples. Of course, fluctuations of the concentration of these values could not be excluded [154].

4.3.3.3 Hereditary congenital cataract

Congenital cataracts in cattle are rare, but the importance for the veterinary practitioner involved into the management of breeding herds and artificial breeding service units increases [16].

Congenital hereditary cataract in cattle was first described by Small in 1919 [155]. He investigated the clinical features of congenital hereditary cataracts in Holstein calves. Already in 1920, the inheritance of congenital cataract in cattle was studied [146]. It could be shown, that the inheritance was autosomal recessive, because both sexes, were equally affected. A Mendelian pattern was evident in which the offspring of two heterozygous carriers in the F1-generation will be carriers, either phenotypically affected or genetically normal homozygous. 75% of the calves will be phenotypically normal. In 1951, Saunders examined a dairy herd, where congenital cataracts in several calves occurred. Through correspondence with the American Jersey Cattle Club registry office, he was able to obtain the extended pedigrees of the last two sires used in the respective herd. Saunders found that if he mated a bull (e.g. bull „S“), carrier of the defective gene, with cows, who are carrier by 50%, a ratio of 7:1 (7 normal calves, one with cataract) is to be expected. He concluded, that if the ratio found in a given herd were higher (for example 7:2), the cause of cataract could not be hereditary [156].

In a herd of grade beef Shorthorn cattle, Leipold (1971) found six blind white calves with multiple ocular lesions (retinal detachment, cataract, microphthalmia, persistent pupillary membranes and vitreous hemorrhage). This was the first report of this congenital syndrome in Shorthorn cattle. The bilateral symmetry of the ocular defects in the calves is, according to Leipold, consistent with a hereditary cause [157].

Gelatt (1971) classified cataracts in cattle into four groups, due to etiopathogenesis: congenital, inflammatory, associated with other eye anomaly, and of undetermined cause. Bilateral congenital cataracts have been described in Jersey, Hereford and Holstein-Friesian breeds [140]. The inheritance was reported to be a simple autosomal recessive trait. The maturation of the cataracts varied in calves from 4 to 11 months of age. Gelatt examined 16 cattle with cataracts at the University of Minnesota and at Kansas State University large animal hospitals, one calf with unilateral and 15 with bilateral cataracts. In two of them, bilateral extracapsular cataract surgery was performed. The eyes were examined with the aid of an indirect ophthalmoscope and a slit-lamp biomicroscope, and were divided into these four groups.

4.4 Bovine Viral Diarrhea

The bovine viral diarrhea virus is a ubiquitous pestivirus with worldwide distribution. It is endemic and transmitted horizontally by direct cow-to-cow contact, as well as vertically from dam to calf. BVDV-induced congenital effects result from fetal infections in the second trimester of gestation. The effects include ocular disorders like cataracts, microphthalmia, optic neuritis and retinal degeneration, but also neurological and other teratogenic defects [158, 159]. Cataracts are described as cortical, while the nucleus is not affected [152, 153]. BVDV is diagnosed by Antigen-detection like immunohistology on cryostat-sections of skin biopsies, PCR or ELISA. Immunohistology is based on antigene detection due monoclonal BVDV-antibodies [160].

4.5 Neospora

Neospora caninum is a well known protozoan parasite causing abortion in cattle and encephalomyelitis in calves after intrauterine infection [161, 162]. The parasite can be diagnosed by PCR, IFAT or ELISA [162-164]. Seropositivity increases the risk for bovine abortion [165, 166]. Neospora caninum is transmitted vertically, so congenital infection is responsible for 80-90% of infections [167, 168]. Infected calves may be born clinically normal or may show neurological signs like paralysis, circling and weakness, and ocular defects, such as blindness and vertical deviation of eyes [166, 169, 170]. The causes of blindness due to Neospora caninum have not been described in detail. Horizontal transmission from the natural host, the dog, is described [171].

In dogs, Neospora caninum can cause multiple ocular lesions, such as retinitis, chorioiditis, iridocyclitis and myositis of extraocular muscles. In the eyes of one dog, Neospora caninum was detected microscopically [172].

Abortions due to Neospora caninum are also described in sheep and horses [173, 174].

4.6 Toxoplasma

Toxoplasma gondii is an obligate intracellular protozoan and therefore depends on the host cell’s metabolism. In humans, T. gondii is the most common cause of chorioretinitis. Prenatal infection carries a higher risk of ocular involvement than postnatal infection [175]. Several cases of congenital cataracts in humans have been attributed to transplacental infection with toxoplasma [176-178]. Screening for IgM and IgG in humans is a standard procedure for preventive measurement in pregnancy [179].

4.7 Mobile base station antennas

4.7.1 Electromagnetic fields

Electromagnetic radiation can be divided into low (> 0-30000 Hertz) and high frequency fields (> 30000 Hz-300 gigaHz). High frequency radiation can generate electromagnetic fields or electromagnetic waves. Exposure to low frequency electric and magnetic fields (EMF) are ubiquitous in modern-day life, because of the use of electricity and its devices. A review about epidemiologic literature on EMF has been published by Ahlbom et al in 2001 [180]. A good overview on the effects of electromagnetic fields in everyday life is given in the report of the Landesanstalt für Umweltschutz Baden-Württemberg in 2004 [181] and also in the report of the Bundesamt für Umwelt, Wald und Landschaft (BUWAL) in 2005 [182].

Mobile phone base stations are radio transmitters that communicate with the users' handset and transmit power levels of ≥ 100W [183]. The number of base stations dramatically increased during the last few years, and so has the exposure to electromagnetic fields. The exposure from base stations shows a diurnal pattern with low levels in the night and two maxima during the day (about noon and in the evenings) [184].

4.7.2 Electromagnetic fields and its impact on cattle

In 2003, Löscher published an study regarding the consequences of electromagnetic fields emitted by mobile phone antennas on health, milk production and behavior of dairy cows [185]. Considerable changes of attitude, fertility problems, abortion, malformation, eye diseases and changes in milk production have been noticed. Ocular diseases included increased lacrimation, conjunctivitis and pruritus. High performance dairy cows seem to be more susceptible to high frequency radiation in consequence of mobile phone antennas [186]. Measured radiation, however, has always been below the limits defined by local legislation. In a study published by Wenzel et al. in 2002, exposed cows showed shortened intercalving times and lowered ruminant frequencies as well as shortened ruminant times. Cortisol concentration in saliva, measured after an ACTH stimulation test, has been elevated in exposed cows [187, 188]. Two research groups measured saliva melatonin concentrations in exposed dairy cows and showed different results [189, 190]. In a Canadian study, EMF (vertical electrical field of 10 kV/m and horizontal magnetic field of 30 μT for 16 hours a day) resulted in a prolonged estrous cycle and a longer duration of the luteal phase in 16 Holstein cows [191].

In summary, electromagnetic fields can act as a stressor, which causes reduced productivity and altered behavior in dairy cattle [185, 187]. The impact of electromagnetic fields on cattle remains an interesting topic; however, there are few scientific studies in this field.

4.7.3 Radiation and cataract

Radiofrequent waves with wavelengths over 1mm have been reported to cause serious retinal damage and cataracts. Already in 1961 Presman noted, that high intensity radiation with radiofrequent waves caused tissue heating and resultant coagulation of lens proteins, whereas low intensity radiation affected lens metabolism [192].

Solar radiation is believed to be one of the most important environmental factors causing senile cataract in humans [193]. Conclusions in this study were that young bovine lenses are less sensitive to UV radiation than 3-year-old lenses and that damage of UV is greater, if the intervals between irradiation stages are too short to permit full recovery of the lens. The cornea blocks most of UV-B radiation (280- 320 nm), but UV-A radiation (320- 400 nm) reaches the lens [194]. Repair of lens proteins in more central regions of the lens does not occur and the lens has no ability to recover from damages accumulated during life. The exact mechanism of UV-A radiation damage to lenses is not yet fully understood. Lee et al. (1999) demonstrated a decreased activity of several antioxidative enzymes (glutathione reductase, glyceraldehydes-3-phosophate dehydrogenase) in human lenses after exposure to UV-A radiation. Glutathione peroxidase and superoxide dismutase showed only small or no changes in their activities [195].

Dovrat (2005) gave evidence, that microwave radiation has a significant impact on lenses. After exposure of bovine lenses to microwaves over 36 h, microscopic and macroscopic changes in the lens remained [196]. Glutathione concentration in rabbit's lenses after exposure to microwaves decreased in proportion to the number of exposures. The absorbed microwave energy seemed to accumulate and therefore lead to changes in the permeability of the capsule and of the membranes of lens fibers. This led to opacities in the lens cortex due to permanent changes in its protein structure [197].

5 Material and methods

5.1 Ophthalmologic examination:

Examination of randomly selected 253 calves from all around Switzerland has been performed in 2005. The calves were examined in abattoirs in Zurich, Basel, Berne, Vrin, Cazis and Brig.

The examination protocol was the following:

1. The ear tag-number of the examined calf was registered for correct identification
2. The eyes of freshly slaughtered calves were examined with a portable slit lamp biomicroscope (Kowa SL 14). For this purpose, a portable black box with shades had been constructed to allow examination of the eye in a dark environment. Dilation of the pupils was not necessary, since the pupils were dilated as a consequence of the captive bolt stunning. If any cataractous lenses were found, digital photographs of the lesions were taken. In addition, cataracts were described and depicted in the examination protocol.
3. Aqueocentesis was performed in each eye with a 22-gauge needle and a 2 ml syringe. The samples were stored in a 1.5 ml tube, immediately frozen in dry ice and protected from light.
4. The aqueous humor samples were then stored at -80°C until further examination.
5. Aqueous humor samples were later investigated for antioxidative enzyme concentration in laboratory.
6. Cataractous lenses were carefully dissected from the globes and submitted for histopathological examination to the Institute of Veterinary Pathology, Vetsuisse faculty, University of Zurich.
7. Skin biopsies were taken from the head of the calves with a 6 mm biopsy-punch. Skin biopsies were analyzed for BVD-MD with immunohistochemical methods.

8. The Institute of Parasitology, Vetsuisse faculty, University of Berne tested the aqueous humor samples for Neospora caninum and Toxoplasma gondii by ELISA.
9. Selenium concentration had been measured by electrothermal atomic absorption spectrophotometry.

Both, male and female calves were examined. Age and sex was not known at the moment of examination.

5.2 Age, sex, breed, mothers age

With the aide of the ear tag-number of the calf and the official registration at the “Tierverkehrsdatenbank”, the respective age, sex and breed of the calf could be determined. Also dam and sir were enclosed. Breed analysis was not possible to be performed due to too much different bulls and too little number of calves per bull.

5.3 Histology

The lenses from 50 calves were examined histological in the Institute of Veterinary Pathology, Vetsuisse faculty, University of Zurich

5.4 BVD immunohistochemistry

Skin biopsies were taken from the cheek region with a 6mm Ø biopsy punch during examination of the calf in the abattoir.

Biopsies were shock frozen in liquid nitrogen. Prior to fixing the biopsies during ten minutes in acetone (at minus 20°C), they were cut into 5-8µm thick cryostat sections. Afterwards, the slides were incubated with H_2O_2 (3% H_2O_2, 0.2% NaN_3) for ten minutes. After incubation with two different monoclonal antibodies (Ca_3 (1:100) and C_{16} (1:100)-Bommeli AG), the EnVision method (DAKO®) was applied while using AEC (Amino-Ethyl-Carbazol) as a chromogen. Between every step, the slides were washed with PBS (phosphate buffered saline, pH 8). With every wash, a positive and a negative control were included.

5.5 Neospora caninum

All bovine aqueous humor specimens were primarily screened for anti-N. caninum antibodies by ELISA as described by Gottstein et al. [163]. ELISA-positive samples were retested by immunoblotting according to Staubli et al (in press) in order to determine the specificity of the reaction. ELISA- and immunoblot-positive samples were subsequently processed by PCR (high pure PCR template preparation kit (Boehringer Mannheim)) for the detection of N. caninum DNA in both the aqueous humor sediment and supernatant [163, 168, 198-200].

5.6 Toxoplasma gondii

A commercially available native, affinity purified P30-antigen (Z.I.SR2B, Avrille, France) was used for the coating of the ELISA plates. Coating and all subsequent test steps were done as previously described for a Neospora-ELISA. The measured absorption values were expressed as percentages of a positive control (antibody units, AU) and the cut-off was calculated from negative controls, as defined by the 3-fold standard deviation of a cluster of 25 negative sera. Samples above the cut-off were considered as positive, those below as negative [163, 201].

5.7 Selenium

Selenium values were determined by electrothermal atomic absorption spectrophotometry with Zeeman background compensation. The adult plasma reference value for selenium is 0.8 +/-0.36 µmol/l [202].

5.8 Laboratory protocols

5.8.1 Introduction

From August 2005 until April 2006 all aqueous humor samples were measured in the laboratory for activities of superoxide dismutase, glutathione peroxidase and catalase. From every calf, two samples, from each eye, were tested and the mean activity was calculated.

The enzyme activities from the aqueous humor of cataractous eyes were then statistically compared to those from healthy eyes.

5.8.2 Superoxide dismutase (SOD)

5.8.2.1 Test kit

The activity of SOD was measured and calculated according to the Fluka®-Testkit.

5.8.3 Glutathions (GSH)

5.8.3.1 Cobas

GSH-Px was measured with Cobas Mira S with the glutathione peroxidase cellular activity assay kit from Sigma®.

5.8.3.2 Calculation

Activity per extract (μmol/min/ml= Units/ml)

$$\boxed{((\Delta A_{blank} - \Delta A_{sample})\, x DF)/6{,}22xV}$$

DF= dilution factor

V= sample volume in ml

5.8.4 Catalase

The activity of Catalase was measured and calculated according to the test kit of Calbiochem®.

5.9 <u>Mobile phone antennas</u>

5.9.1 Introduction

Swiss legislation requires that cattle movements be registered, which allows the tracking of all bovines until slaughter. The individual ear tag-number of the examined calf allowed the follow-up of the addresses of every calf's owners. Via Twix route, the coordinates of every barnyard could be determined, from conception until slaughter of the calf. Because ocular organogenesis during fetal development takes place during the first and second trimester of gestation, this time period was of special interest.

5.9.2 Analysis

The relationship between the geographical position of the farms (n=362), on which the dams and calves were housed, and the strength and distance to mobile telephone BS within a 10 km radius (n=608’141 GSM or UMTS transmission units on 10’716 locations) was determined. The Swiss Federal Office for Communication provided the information about the location of mobile phone base stations and their respective frequency (GSM and UMTS) and strength (V/m). The base stations were grouped with respect to the distance between the barnyards and the BS.

0- 99m (5 BS, smallest distance 6m)
100- 199m (9 BS)
200- 299m (9 BS)
300- 399m (13 BS)
400- 499m (21 BS)
500- 999m (124 BS)
1000- 1999m (263 BS)

The maximum theoretical field strengths of the closest BS with omni-directional radiation without topographical correction were >1 V/m (40 locations), 0.5- 0.99 V/m (121 locations), 0.1- 4.99 V/m (190 locations) and <0.1 V/m (11 locations). All base stations within a distance of 2000 m from the actual position of the barnyard were included in the study. For calculation of the total field strength, all GSM and UMTS BS within a radius of <10 km were included.

Time period of the second and the third trimester of pregnancy were combined because only a few calves moved to another barnyard during this period. Those calves were excluded from statistical analysis.

5.10 Statistical methods:

Data were analyzed using factorial analysis of variance, logistic regression, χ^2-test or simple linear regression modeling to calculate dependency between biochemical data and distance to nearest BS. Because the numbers of cases with severe nuclear cataracts were small, results were controlled not only for significance but also for power. Only results with $p \leq 0.05$ and power ≥ 0.8 are reported as significant. Accuracy of obtained results was tested using Hosmer-Lemeshow statistics [203].

6 Results

6.1 Ophthalmologic examination

6.1.1 General

Over a period of one year, 253 calves from both sexes were ophthalmologically examined in several abattoirs all over Switzerland. Thereof, 81 calves had various lenticular opacities. 96.8% of the cataracts were bilateral. Cataracts were classified into categories (Figure 4 and Figure 5). 9 (3.6%) of the 253 calves had severe cataracts with fully opacified lenses (Figure 6, Figure 7 and Figure 9). 13 (5.1%) had moderately developed cataracts; in 25 (9.9%) cases, the opacity was detectable centrally beginning in the lenses nucleus. A condensed posterior nucleus border (Figure 8) was found in 15 (5.9%) calves, in 2 (0.8%), the whole posterior half of the nucleus was cloudy. 11 (4.4%) calves showed condensed nucleus borders, whereas 6 (2.4%) calves showed focal opacities in the lenticular nucleus.

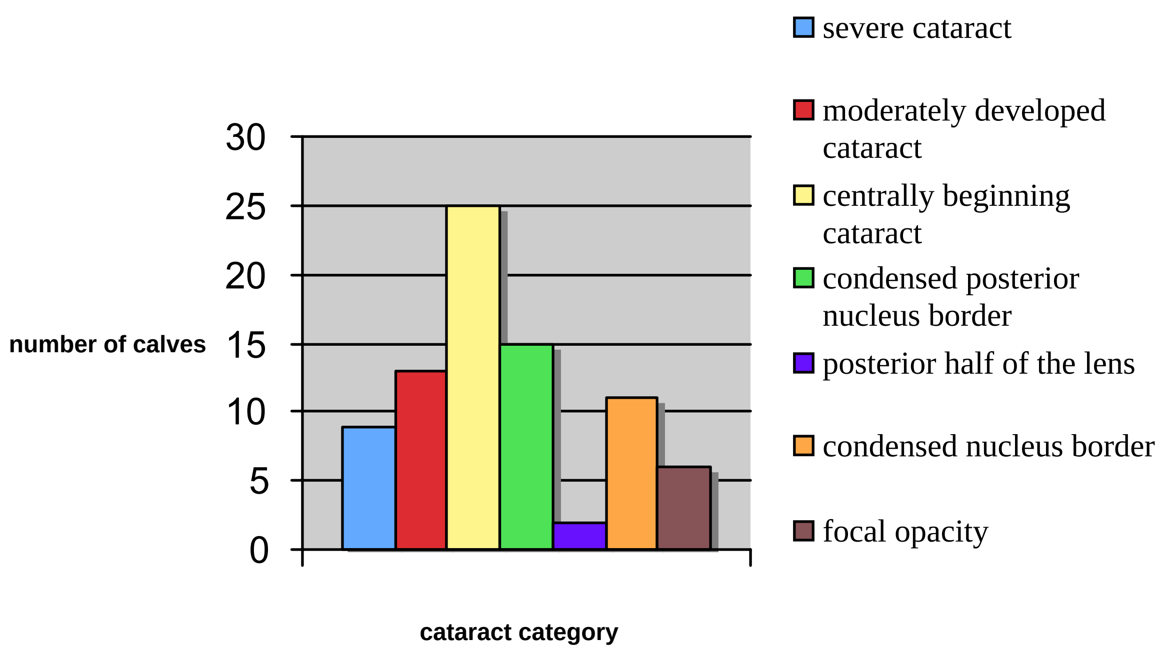


Figure 4: Cataract categories

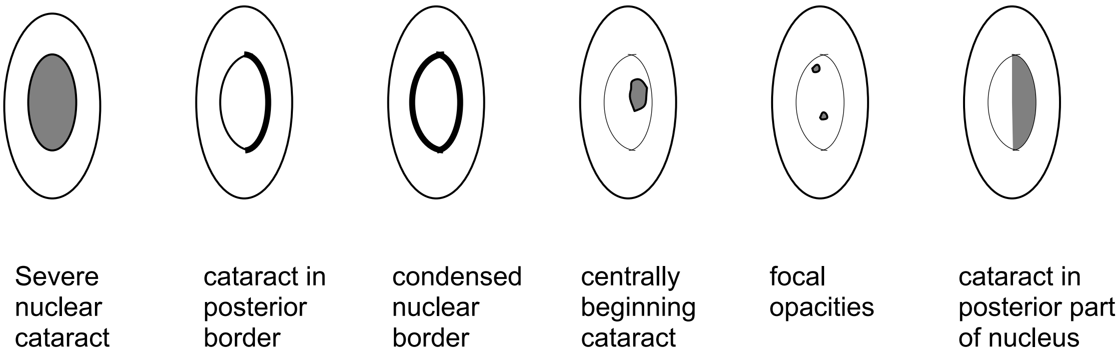


Figure 5: Schematic drawings of categories of nuclear cataracts

Figure 6: severe nuclear cataract

Figure 7: severe nuclear cataract

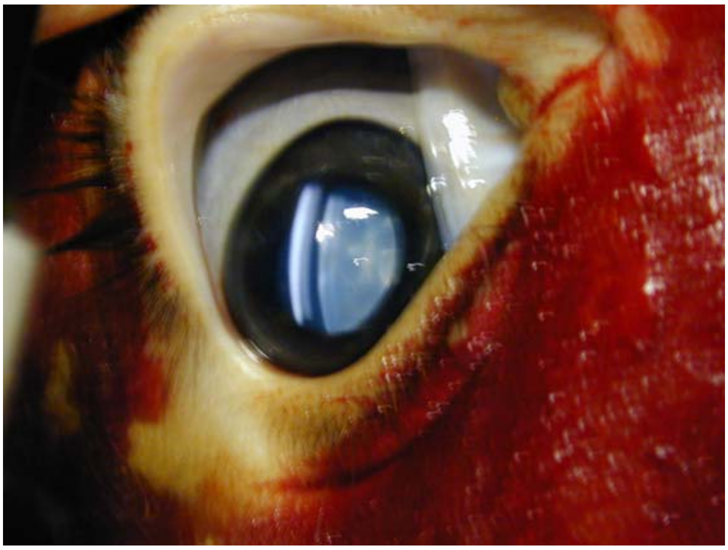


Figure 8: cataract in the posterior nucleus border of the lens

245 (96.8%) calves showed bilateral nuclear cataracts. In 8 calves (calves number 91, 122, 141, 144, 160, 179, 180 and 192), the nuclear cataracts were unilateral.

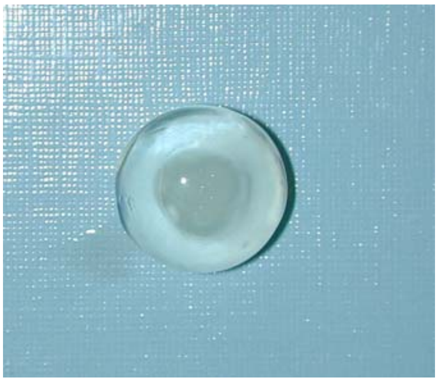


Figure 9: Lens with severe nuclear cataract

The several categories were grouped as following for statistical analyses (Table 3):

Table 3: grouping of cataracts

Diagnosis	**Category**	**N**
Dense nuclear cataract	severe	9
Central nuclear Opacity	mild	6
Focal peripheral nuclear opacity	mild	22
Opacity of the posterior border of the nucleus	mild	15
Opacity of the posterior part of the nucleus	mild	2
Moderately developed cataract	moderate	13
Not classified cataract	mild	1
Condensed border between nucleus and cortex	mild	11
No cataract	no	174
Total		253

6.1.2 Sex

Male calves were more often affected than female calves (Figure 10). 156 male calves were examined; thereof 57 calves (36.5% of the male calves) had shown clinical cataracts. Of 95 female calves, only 24 (25.3% of the female calves) showed nuclear cataracts. The results were statistically not significant, but showed a tendency (p-value=0.06). Two calves could not be included into statistical analysis, because of invalid ear tag.

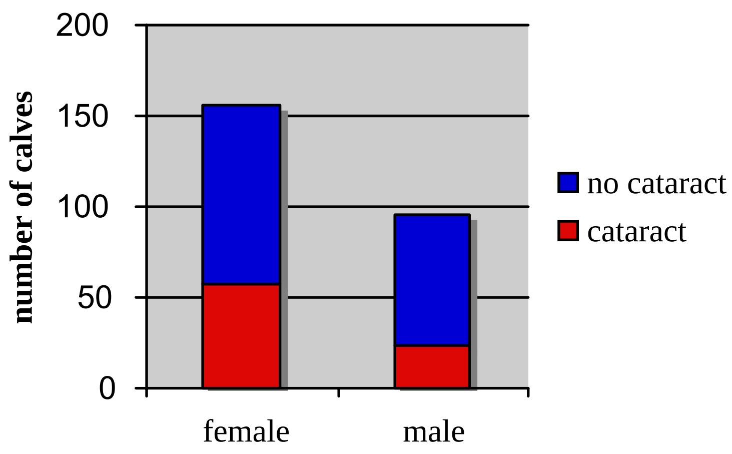


Figure 10: Sex

6.1.3 Age of calves

The youngest calf was 83 days old, the oldest calf, which was examined, had an age of 370 days (

Figure 11). The mean age was 146 days. In two calves, the ear tag was invalid.

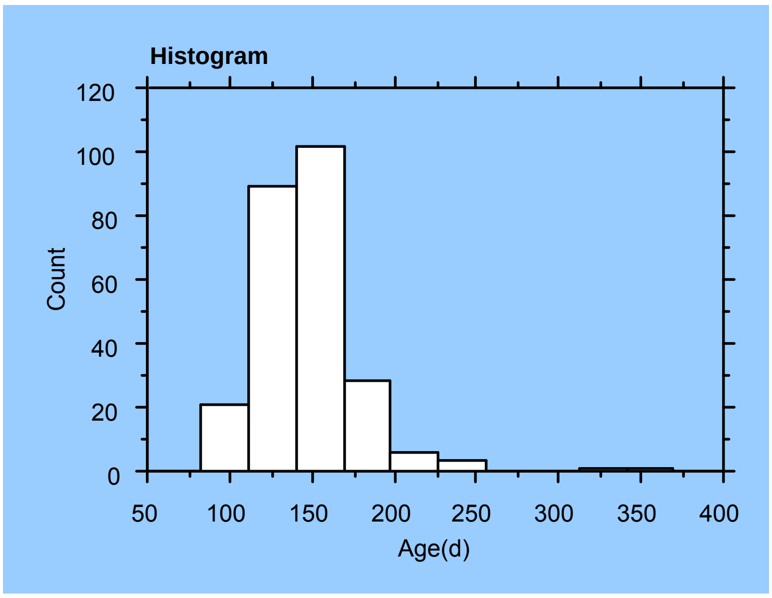


Figure 11: Histogram calves' age

6.1.4 Breed

In terms of the calves' breed, no statistically significant difference could be found (p= 0.6). All breeds were affected with nuclear cataracts (Table 4 and Figure 12). Two calves could not be included into statistical analysis, because the ear tag was invalid.

Table 4: Breed

Breed	Animals examined	Cataracts found	% of examined calves
Mixed breed, divers	127	39	30.7% of mixed breed
Brown Swiss	41	15	36.6% of Brown Swiss
Red Holstein	53	15	28.3% of Red Holstein
Holstein	30	12	40.0% of Holstein

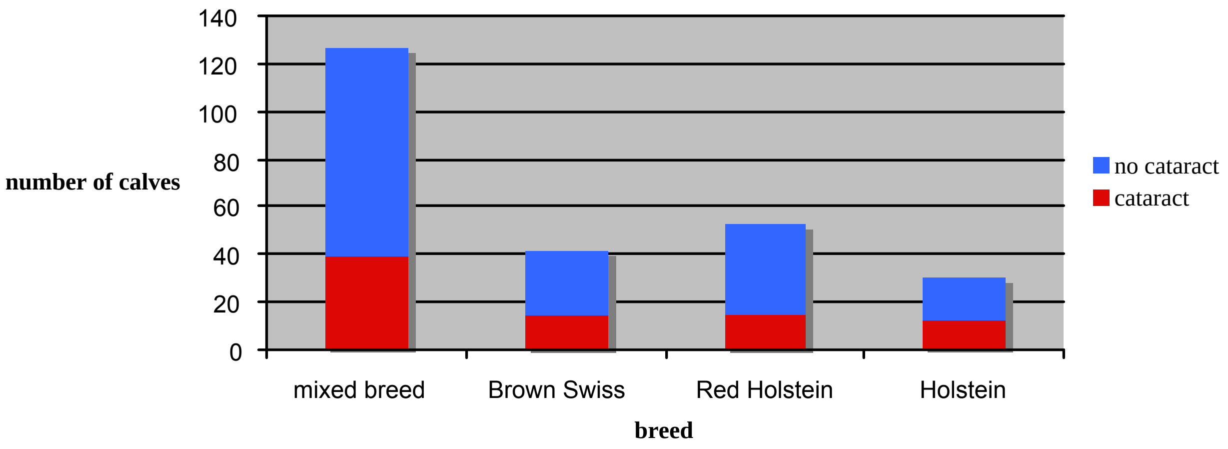


Figure 12: Breed

6.1.5 Age of mother

Researches about the age of the mothers at the time of insemination brought no statistical significant results (p= 0.9). The calves of old cows and of young cows had an equal degree of probability to be born with nuclear cataracts.

6.1.6 Provenance of the calves

Calves from 22 different cantons of Switzerland were examined. No statistical relevant conclusion could be made about the frequency of cataract calves in every canton (Table 5 and Figure 13).

Table 5: Provenance of the calves

Canton of provenance of the slaughtered calf	Number of slaughtered calves in respective canton in 2005	Number of examined calves in respective canton	% of calves examined in respective canton	Number of calves with cataracts found in respective canton
AG	24983	39	0.16%	17
AI	4053	2	0.05%	0
AR	7141	0	0	0
BE	51429	31	0.06%	9
BL	5319	6	0.11%	3
BS	69	0	0	0
FL	243	0	0	0
FR	21444	18	0.08%	4
GE	74	0	0	0
GL	2200	3	0.14%	1
GR	13010	13	0.10%	3
JU	5866	3	0.05%	1
LU	36032	32	0.09%	10
NE	4428	2	0.05%	0
NW	2685	2	0.07%	2
OW	2276	0	0	0
SG	33623	17	0.05%	4
SH	2836	1	0.04%	0
SO	9971	3	0.03%	1
SZ	11802	3	0.03%	1
TG	16192	15	0.09%	4
TI	1379	7	0.51%	2
UR	4556	10	0.22%	5
VD	12163	2	0.02%	1
VS	3070	1	0.03%	0
ZG	5671	1	0.02%	0
ZH	20032	42	0.21%	13
total	302549	253	0.08%	81

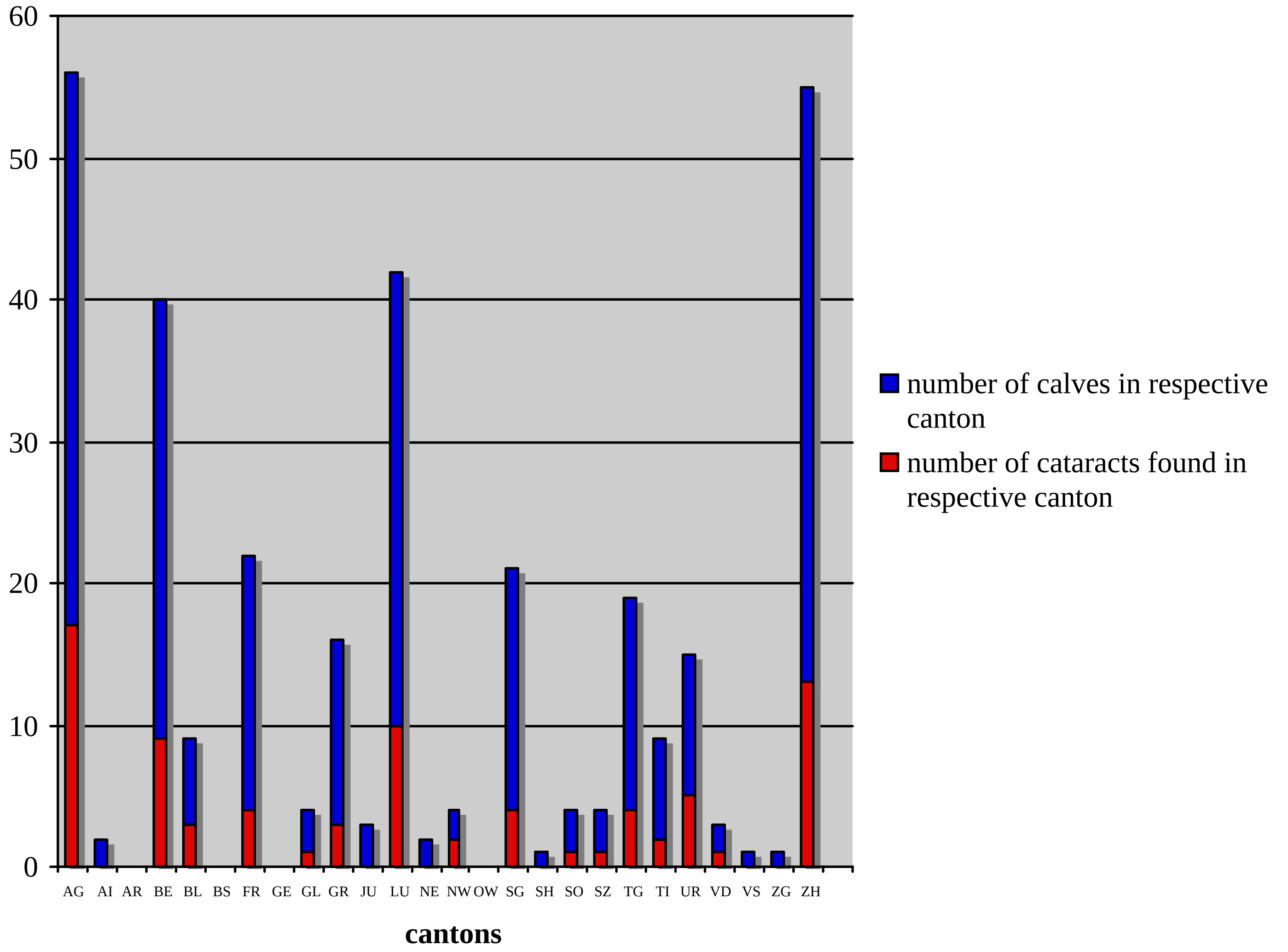


Figure 13: Provenance of calves

6.2 <u>Histology</u>

50 lens pairs with clinically diagnosed nuclear cataract have been examined histologically. Of these 100 lenses, 62 (62%) had histologic evidence of cataract. 38 lenses showed no histologic signs of nuclear cataract in the sections examined.

Histological findings were:

- Loss of organization of lens fibers. Nucleus/cortex are not recognizable any more.

- Degeneration and swelling of lens fibers in the nucleus and the nucleus border with homogenization of the fiber material
- Persistence of cell nuclei or remains of nuclei in the lenticular nucleus
- Focal accumulation of homogenous eosinophilic material, interfibrillarily disposed in the nucleus or, often seen, in the nuclear border, mostly surrounded of swollen lens fibers
- Material with a foamy appearance in the nucleus

Clinically severe cataracts were detected histologically in a higher percentage than focal cataracts.

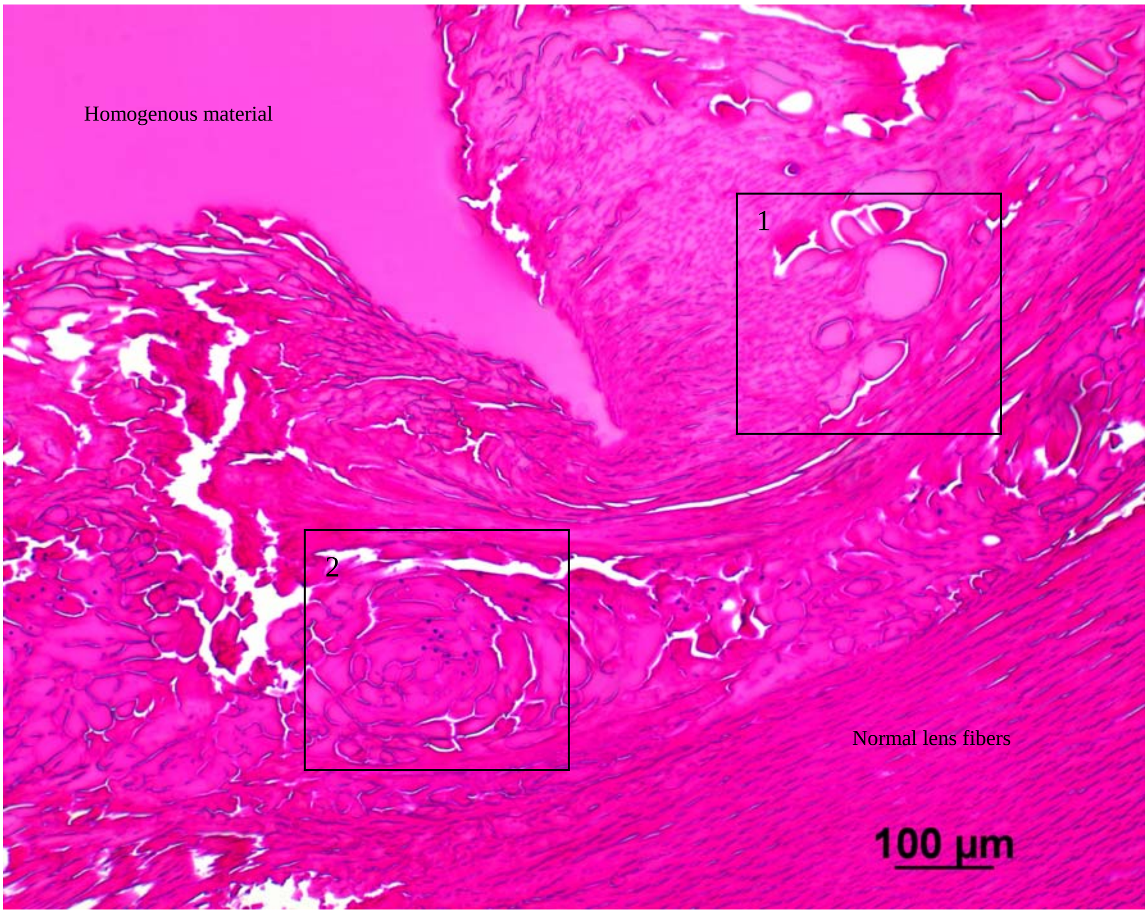
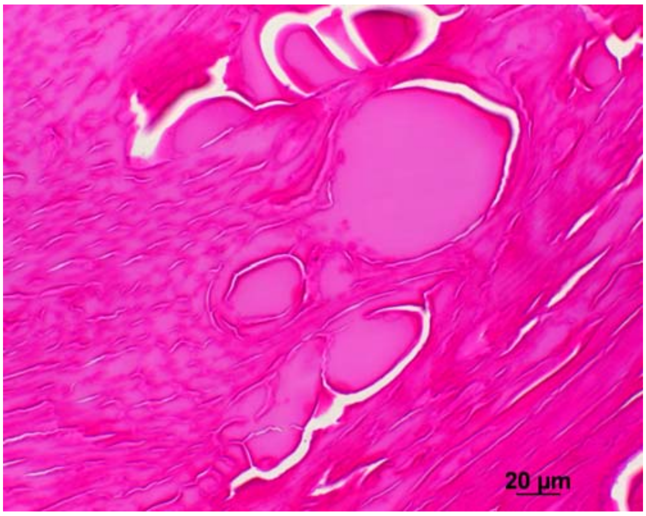


Figure 14: severe nuclear cataract

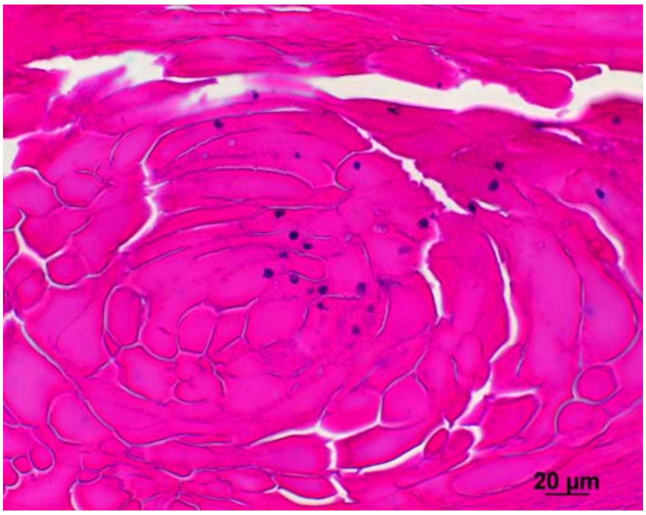
Section 1

Swelling and disaggregation of lens fibers



Section 2

swollen fibers with nuclei



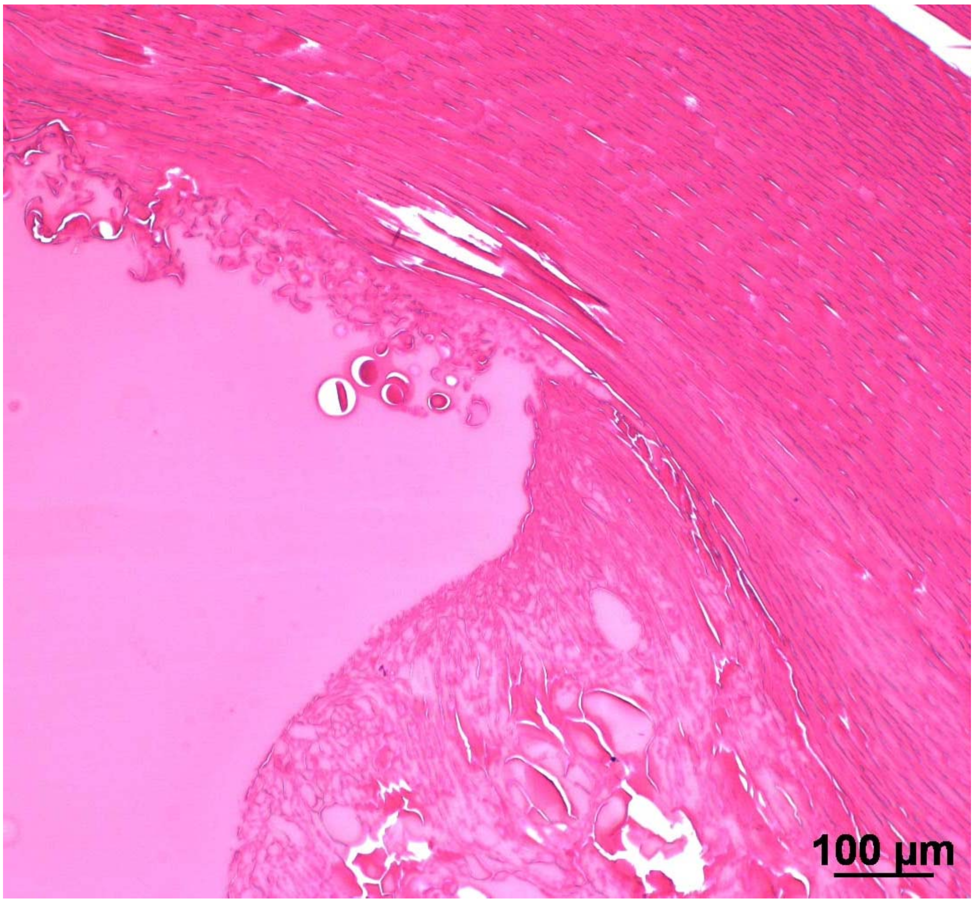


Figure 15: severe nuclear cataract: homogenization of lens fiber material

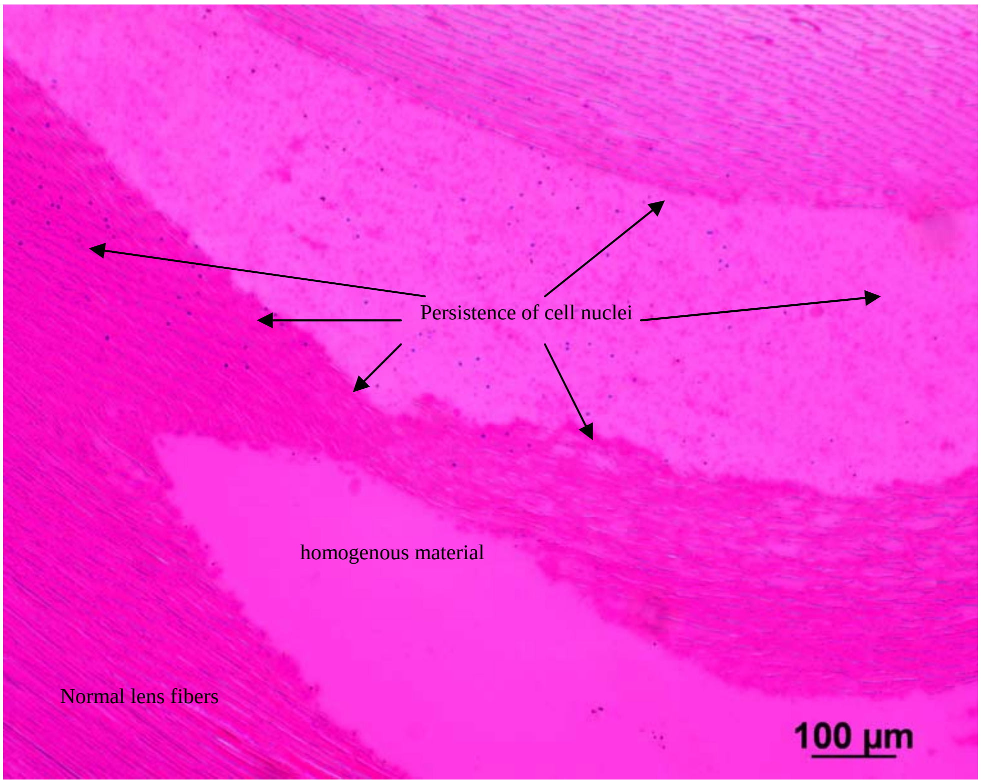


Figure 16: condensed nucleus border

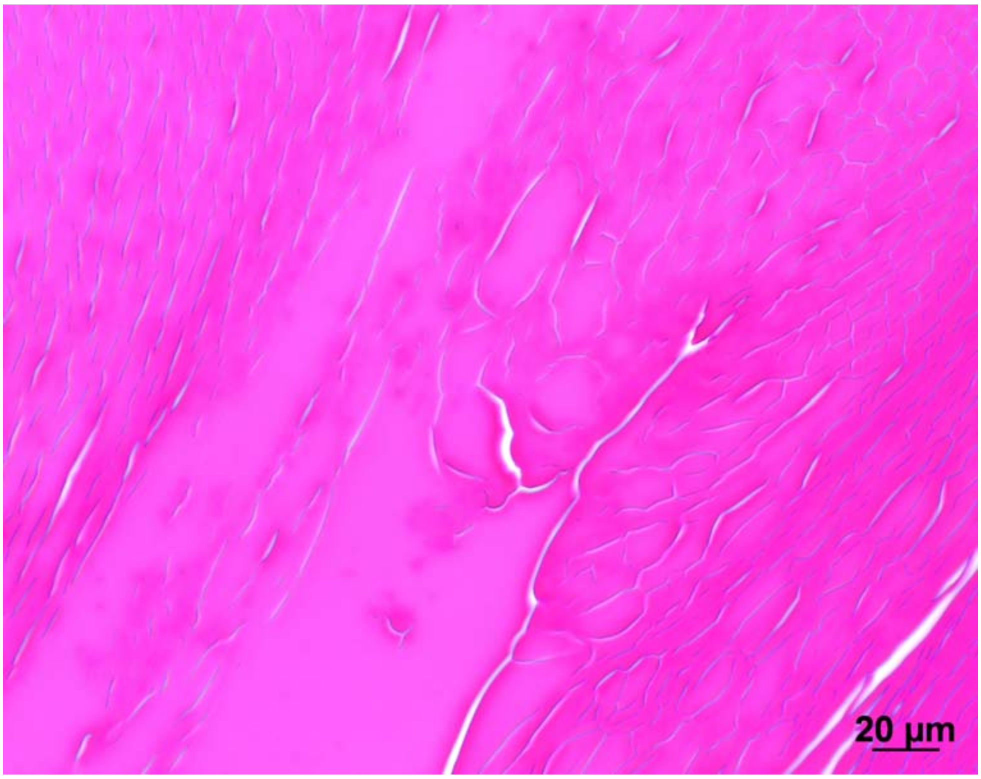


Figure 17: beginning nuclear cataract, swelling of lens fibers and homogenization of lens tissue

6.3 Laboratory results

After statistical comparison between the enzyme activities in aqueous humor from cataractous eyes and healthy eyes, the following coherences resulted:

6.3.1 Glutathione peroxidase

Glutathione peroxidase had a mean value of 28.260 (standard error of ± 2.688) in aqueous humor samples with no cataract and 21.132 (± 1.733) in samples with cataract. The difference of the enzyme activity was statistically significant (p= 0.03).

Correlation between samples from calves with severe and moderate cataracts and from calves without cataracts brought no statistical significant results (p-value= 0.06).

6.3.2 Catalase

Catalase activity had a mean value of 10.661 (± 0.595) in samples without cataract and 9.437 (± 0.537) in samples with cataract. The difference was not significant, but samples with cataract had a tendency for lower activity than those without cataract (p= 0.13).

The activity of catalase in aqueous humor samples of calves with severe or moderate cataracts showed no statistical significant, but a tendency for lower activity in calves without cataracts (p= 0.14).

6.3.3 Superoxide dismutase

The activity of superoxide dismutase had a mean value of 53.765 (± 1.742) in the aqueous humor of healthy eyes and 53.742 (± 1.704) in eyes with cataract. There was no statistical difference (p= 0.99).

6.4 BVD

Four calves with the numbers 33, 50, 80 and 216 were tested positive for BVD by immunohistochemistry. All four affected calves had no nuclear cataracts. All other skin biopsies tested negative.

6.5 Neospora and Toxoplasma

All aqueous humor samples tested negative for Toxoplasma gondii (ELISA). 253 calves tested negative for Neospora caninum with ELISA, one was tested mildly positive. This sample was investigated with PCR for Neospora caninum and was negative.

6.6 Selenium

Selenium values were below the detection level, even if no dilution was performed. Therefore no further investigations had been made.

6.7 Heredity

The database of 251 calves could be used for heredity analysis. From two calves, the ear tag number was not usable. For 251 calves, 143 different bulls were used for artificial insemination. The highest insemination rate by one bull was 28. The mean insemination rate by one bull was 3.407 with a median of 1. Therefore no further statistical analysis for heritability was performed.

6.8 Mobile phone antennas

There was an association between the strength of mobile phone antennas and nuclear cataract in veal calves, first seen in the first trimester of gestation (Factorial ANOVA- test, Table 6). An association between oxidative stress and nuclear cataract in veal calves was first seen in the second trimester (linear regression, Table 7). The number of antennas within 100 to 199 meters of a pregnant cow affected oxidative stress only in the first trimester of gestation. The strength of association by means of odds ratio is given in Table 8 (logistic regression). Oxidative stress as evidenced by decreased GPx activity was significantly elevated (OR per kilometer: 0.80, confidence interval 95% 0.62, 0.93). Hosmer-Lemeshow statistics showed an accuracy of 100% in negative cases and only 11.11% accuracy in positive cases due to low number of calves with severe cataracts.

Table 6: Factorial ANOVA-test for association between nuclear cataract in veal-calves and mobile telephone antenna base stations

Cases/ controls	*Factors tested*	*1. Trimester*		*2./3. Trimester*		*Birth*	
		P-value	Power	P-value	Power	P-value	Power
Severe cataracts/all others	Strength next BS[1]	0.0001	0.986	0.0002	0.982	0.0007	0.946
	Strength next BS per distance	<.0001	0.995	<.0001	0.994	<.0001	0.994
	Strength total BS[2] per distance	<.0001	0.994	<.0001	0.992	<.0001	0.988
Severe cataracts/no cataract	Strength next BS	0.0013	0.92	0.0013	0.919	0.0047	0.828
	Strength next per distance	0.0007	0.95	0.0007	0.946	0.0008	0.943
	Strength total per distance	0.0007	0.949	0.0008	0.944	0.0012	0.926

[1]: Next BS within 2 km radius
[2]: All BS within 10 km radius

Table 7: Linear regression association between distance to mobile telephone antenna base stations and oxidative stress in nuclear cataract in veal-calves (Power was set at 0.8)

Model	Factors tested for association	1. Trimester	2./3. Trimester	Birth
		P-value	P-value	P-value
Severe cataracts/all Others	GPx to distance next BS	0.8644	0.0236	0.0323
Severe cataracts/no cataract	GPx to distance next BS	0.6391	0.0016	0.0058
all cataracts/no cataract	GPx to distance next BS	0.8644	0.0236	0.0323
severe & moderate cataracts/all others	GPx to distance next BS	0.8644	0.0236	0.0323
severe & moderate cataracts/ no cataract	GPx to distance next BS	0.7525	0.0018	0.0058
	GPx to no. of antennas within distance 100- 199 meters	0.0002	0.372	0.3556

[1]: Next BS within 2 km radius
[2]: All BS within 10 km radius

Table 8: Odds ratio (OR) from logistic regression model for mobile telephone antenna base stations in nuclear cataract in veal-calves

Cases/ controls	Factors tested for association	1. Trimester		2./3. Trimester		Birth	
		OR	CI 95%[3]	OR	CI 95%[3]	OR	CI 95%[3]
Severe cataracts/ all others	Strength next BS[1]	1.74	1.16, 2.63	1.73	1.14, 2.61	1.67	1.11, 2.52
	Strength all BS[2]	1.23	1.01, 1.51	1.24	1.06, 1.52	1.20	0.97, 1.49
Severe cataracts/ no cataract	Strength next BS	1.64	1.09, 2.45	1.63	1.09, 2.44	1.57	1.05, 2.36
	Strength all BS	1.21	0.99, 1.48	1.22	1.00, 1.49	1.18	0.95, 1.46

[1]: Next BS within 2 km radius
[2]: All BS within 10 km radius
[3]: confidence interval 95%

7 Discussion

7.1 General

7.1.1 Index case

Index case for this study was a Swiss dairy farm, where 25% of the newborn calves showed severe nuclear cataracts with a temporal coincidence with the deployment of a mobile telephone base station on the farm. In this case, all known etiologies for nuclear cataracts in calves could be ruled out.

Since the prevalence of nuclear cataract in calves in Switzerland was unknown, the present study was initiated.

7.1.2 Prevalence and diagnosis

In several earlier studies about prevalence of cataracts in cattle [139-142], the prevalence of diagnosed cataracts is very different and the examination-technique for the diagnosis is not described. Slit lamp biomicroscopy, the diagnostic method in the present study, is capable to detect even mild or focal lenticular opacities, which shows the detection-bias of this diagnostic method compared to simple focal light sources without amplification.

In the present study, diagnosis resulted from examination with slit lamp biomicroscopy with 10x magnification in a shaded box, so even minute lenticular opacities could be recognized. In one study, cataracts have been diagnosed histologically [144]. We could show in the actual study, that histological diagnosis of cataracts has a low sensitivity of 68% with clinical diagnosis as a gold standard. A cataract is an optic phenomenon of refraction, which appears often not to be recognizable in histological examination.

7.1.3 Etiology

This study failed to reveal any infectious etiology for nuclear cataracts in veal calves. Bovine virus diarrhea (BVD), Neospora caninum and Toxoplasma gondii could be ruled out as causes of nuclear cataract in the examined calves. This result was expected regarding BVD, because BVDV-related cataracts are described to be cortical [152, 153].

Results regarding the mobile phone antenna issue are described below. The question remains, whether the prevalence of cataract in veal calves is higher than in the average calf population. Does the fact, that veal calves are only fed with milk and straw enhance the risk for cataract? On the other hand, nuclear cataracts develop during early gestation, so environment factors of the calves seem to be less important. If feeding milk or milk replacer (orphan diets) was the reason for cataract, the opacities wouldn't be recognizable already at birth and the majority of the veal calves would be affected. No feed analysis has been performed. Crucial food content seems to be selenium due to its influence on the oxidation status of blood. To eliminate any selenium deficiency to be the reason for elevated oxidative stress, reduced selenium concentration in the aqueous humor should be excluded. Our laboratory tests failed to eliminate this confounder because of too low sensitivity of the test method.

It is also unknown whether calves with cataracts appear weaker and go to the abattoir because they drop behind in weight gain and growth. This seems unlikely, since the majority of veal calves are selected for that purpose at a very early age.

Heredity as etiology for nuclear cataract in calves failed to play any role in the present study, because of the low number of cases. For further investigations concerning this subject, more calves should be examined and pedigree analysis should be done.

Neither comparison between the calves' mothers' age at the time of insemination and the probability of nuclear cataract resulted in any statistical significant relevance [141].

7.1.4 Oxidative stress

Statistically significant decreased activity of glutathione peroxidase in aqueous humor of eyes with cataract allows the suggestion of the eye being under oxidative stress. The activity of catalase is also low, but not statistically significant. Superoxide dismutase does not seem to play a role. One reason could be, that superoxide dismutase is less susceptible to the respective noxa. Superoxide dismutase produces hydrogen peroxide in the mitochondria. Maybe transferrin is able to bind iron and therefore can inhibit iron to react with hydrogen peroxide. So the production of free radicals due to superoxide dismutase could be diminished. Low GPx activity leads to accumulation of H_2O_2, which is the major oxidant contributing to cataract [55].

A reason for low GPx activity and oxidative stress in the lens could also be selenium deficiency. Is glutathione peroxidase activity lower in cataract eyes because of selenium deficiency or because of high oxidative stress and therefore a depletion of glutathione peroxidase? This question cannot be answered because of the lack of selenium measurements in the present study.

7.2 Mobile phone antennas and cataract

7.2.1 General

There is a high public concern about possible deleterious health effects of electromagnetic fields from mobile phone communication systems, particularly from mobile phone antennas, whose number increased heavily during the last few years.

The concern about health risks from living near to transmitters is directed towards subjective symptoms such as fatigue, sleep disturbances and frequent headache. Therefore, results of epidemiologic studies to date gave no consistent or convincing evidence of a causal relation between exposure to electromagnetic fields and any adverse health effect. These studies have too many deficiencies to rule out an association [184].

Refinement of exposure assessment is critical to improve the quality of epidemiologic studies. A key element may be a dosimeter that is capable of monitoring individual exposure. Ideally, the dose, time pattern, and frequencies (wavelengths) of exposure from all key sources should be measured for each point or individual in the study [184].

Most epidemiologic studies about the effects of radiofrequency fields are flawed due to cost and/or time restrictions. The relation between exposure and distance from the antenna is very complex, especially in urban areas. Aspects to consider are:

- Mobility of individuals
- Accumulation of fields from several antennas, which cover the same area
- Topography, which changes the intensity of the electromagnetic field with increasing distance from the antenna

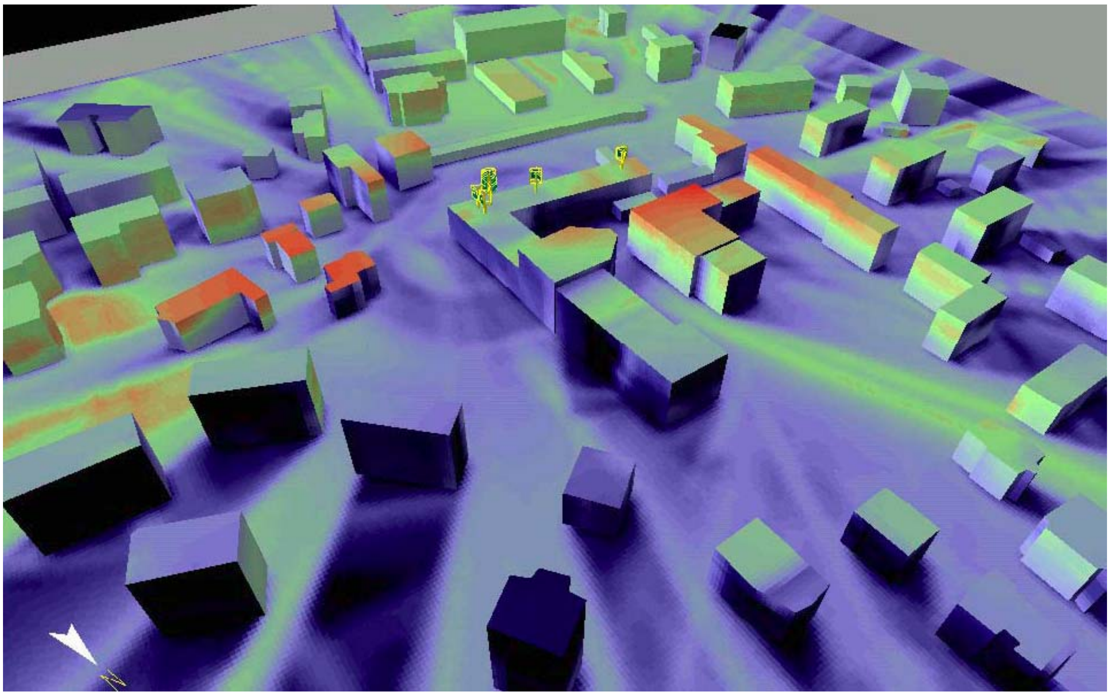


Figure 18: Radiation of a mobile phone antenna in urban area (from http://www.bakom.ch)

7.2.2 Own investigations

Due to the official registration of movement of each farm animal, we were able to link its position from breeding to stockyard with a possible influence of electromagnetic fields on nuclear cataract in veal calves. Since the embryonic development of the eye falls into the organogenesis period of the first trimester of gestation, this time was particularly emphasized [14].

In the present study, the risk for development of nuclear cataract in calves during organogenesis may be associated to mobile telephone antenna base stations.

Due to wave nature of electromagnetic fields and topographic effects, the results should be interpreted with caution. The field strength of electromagnetic waves can vary over short distances [192]. In addition, the exposure of pregnant cows and calves to electromagnetic fields varies because they move about the boundaries of the barnyard. Nevertheless, conservative calculations that excluded distance revealed a significant association. Although results are significant ($p<0.05$) with high power (>0.8), a back calculation from BS distance or field strength to risk of nuclear cataract is not possible, because of too small a number of severe nuclear cataracts. The presented results have to be viewed critically. Because of the cross-sectional study design, the dependent variable nuclear cataract was measured at the end of the calf's life and the independent variable exposure to electromagnetic waves was measured at the three different time periods. Thus, it was not possible to specify the exposure to a particular time period. Furthermore, biases such as topographical disturbances or different mobile telephone frequencies could not be ruled out. Further studies on the influence of electromagnetic fields have to take into account the embryonic stage of the animal or person at risk. Also the possible accumulative effect of radiation due to radio, television, microwave or high voltage lines should be considered. Especially the influence of radio transmitters, which have very high radiation power, has to be considered [204].

The lack of adequate dosimetry is a weak point of the present study and a direct result of the cross-sectional study design. The decision to work with averages, concerning field strength and location of the animals is a well-known source of error in this study. In any way, the movement of calves and their dam under Swiss condition is much less than the one of human beings. But due to their limited possibility of free movement our assumption of an average radiation may be acceptable.

Another fact to be considered is the direction of the radiance of the antenna. There are base stations radiating omnidirectional, others are radiating sectional and with a certain vertical angle.

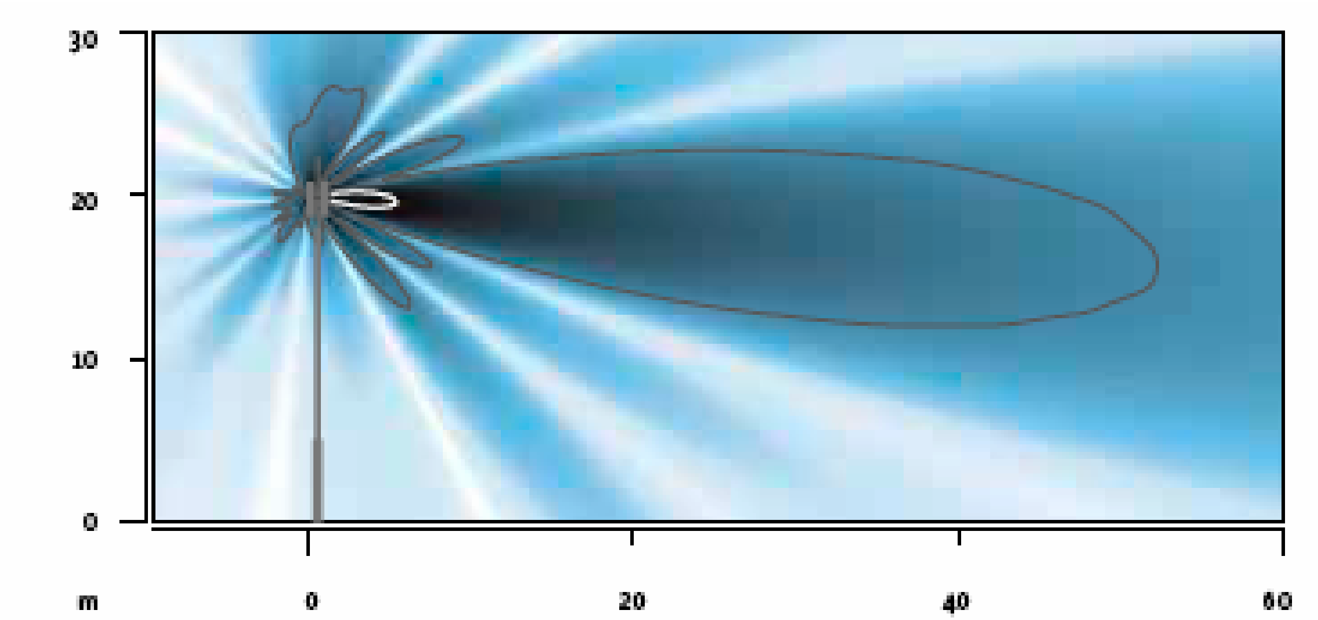


Figure 19: Direction of the radiation of a mobile phone antenna (from http://www.bafu.ch)

The actual transmitting power of the BS was reported to Bakom on a monthly basis by the operating company and not controlled by Bakom. Therefore the data from Bakom have to be considered itself with a certain error. Also the radiation was considered to be omnidirectional and sectional radiation was not modeled in our calculation, since the direction was not available for all antennas.

Oxidative stress may also be related to the presence of a mobile telephone BS and to total exposure to electromagnetic fields. Heating of cells and tissues from electromagnetic exposure is a known phenomenon. Laboratory studies suggest that biologic effects can be caused by temperature rises in tissue that exceed 1°C above their normal temperatures [205]. The lens is highly sensitive to heat and damage can occur from even a single acute exposure. But in Switzerland, the exposed radiations of mobile phone antennas are far beneath legal limit so this etiology for cataract can be excluded. However, it is important to know, that high rates of physical activity and warm and humid environments will reduce tolerance to additional heat loads [184]. Our results suggest that heat is not the only factor of concern but also oxidative stress and many others yet not mentioned factors. This also has to be taken into account in future studies.

Experimental designs of further studies should eliminate most of these biases and would therefore be preferable. Although the base stations, due to their weak electromagnetic fields are considered unlikely causes of deleterious health effects, the present study suggests that this possibility should be taken seriously.

8 Literature

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